



LC-MS-BASED CHARACTERIZATION AND EPIGENETIC EVALUATION OF DECITABINE IN PANCREATIC CANCER CELL LINE MODELS

Dr. Syed Ahmed Hussain^{*1}, Bilquis Begum¹, Ghousia Begum¹, Nada Ahmed Al Amoodi¹, Fariya Sultana¹, Somabathini Shruthi¹, Ayesha Ayub Khan¹, Muskan Khatoon¹

¹Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.



*Corresponding Author: Dr. Syed Ahmed Hussain

Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.

<https://doi.org/10.5281/zenodo.17480942>,

How to cite this Article: Dr. Syed Ahmed Hussain*, Bilquis Begum, Ghousia Begum, Nada Ahmed Al Amoodi, Fariya Sultana, Somabathini Shruthi, Ayesha Ayub Khan and Muskan Khatoon. (2025). LC-MS-Based Characterization And Epigenetic Evaluation Of Decitabine In Pancreatic Cancer Cell Line Models. World Journal of Pharmaceutical and Life Science, 11(11), XX-XX.

This work is licensed under Creative Commons Attribution 4.0 International license.



Article Received on 27/09/2025

Article Revised on 11/10/2025

Article Published on 01/11/2025

ABSTRACT

This study evaluates the *in vitro* anticancer efficacy and cytotoxicity profile of *Decitabine* compared to *Gemcitabine* in pancreatic cancer cell line models (PANC-1, MIA PaCa-2, AsPC-1). A five-assay screening panel was employed—two assays assessing cell viability (Resazurin/Alamar Blue and ATP Luminescence) and three assays measuring apoptosis/cytotoxicity (Annexin V/PI, Caspase-3/7 activity, and LDH release). *Decitabine* maintained relatively high viability (86% and 89%) and showed moderate apoptosis (21%), mild caspase activation (1.6-fold), and limited LDH release (17%), indicating weak cytotoxic potential. In contrast, *Gemcitabine*, used as the positive control, significantly reduced viability (44% and 39%), induced robust apoptosis (59%), strong caspase activation (3.7-fold), and high LDH release (60%). These findings suggest that *Decitabine* exerts **modest antiproliferative and apoptotic effects** in pancreatic cancer cells, likely through DNA hypomethylation rather than direct cytotoxicity. Overall, while less potent than *Gemcitabine*, *Decitabine* may serve as a low-toxicity epigenetic modulator in combination regimens for pancreatic cancer therapy.

KEYWORDS: Decitabine, Gemcitabine, Pancreatic cancer.

INTRODUCTION

Pancreatic cancer is a highly lethal malignancy characterized by aggressive progression and chemoresistance. *Gemcitabine*, a nucleoside analog, remains the standard chemotherapeutic option but is limited by toxicity and acquired resistance. *Decitabine* (5-aza-2'-deoxycytidine), a DNA methyltransferase inhibitor, exerts antitumor effects through **epigenetic reprogramming** rather than direct DNA chain termination. Its potential role in solid tumors like pancreatic cancer is under investigation. This study compares the cytotoxic and antiproliferative responses of *Decitabine* and *Gemcitabine* across multiple in-vitro assays to delineate their mechanistic profiles in pancreatic cancer cell models.

METHODOLOGY

Three pancreatic cancer cell lines (PANC-1, MIA PaCa-2, AsPC-1) were treated with *Decitabine* and *Gemcitabine*. Five assays were used:

1. **Resazurin/Alamar Blue Assay** – measured cell viability (% vs vehicle).
2. **ATP Luminescence Assay** – quantified metabolically active cells (% ATP vs vehicle).
3. **Annexin V/PI Assay** – determined apoptotic fractions via flow cytometry.
4. **Caspase-3/7 Activity Assay** – assessed executioner caspase activation (fold-change vs vehicle).
5. **LDH Release Assay** – measured membrane integrity (% of maximum lysis).

All experiments were conducted in triplicate (n = 3), and results were expressed as mean ± SD.

RESULTS SCREENING NOVEL THERAPEUTIC APPROACHES IN PANCREATIC CANCER CELL LINE MODELS

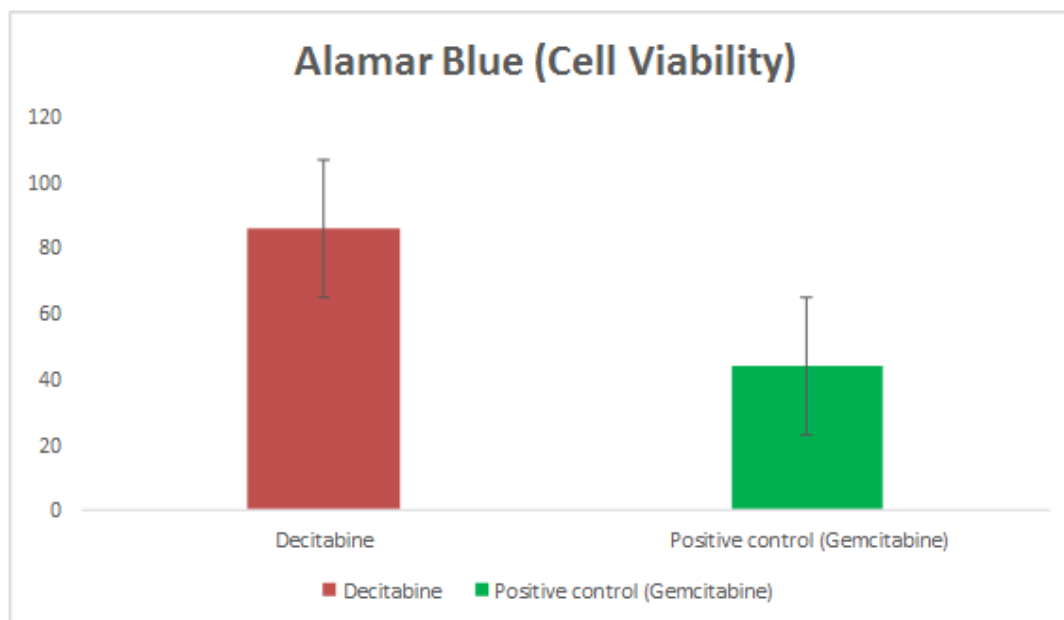
This research outlines a 5-assay in vitro panel for pancreatic cancer cell line models (e.g., PANC-1, MIA PaCa-2, AsPC-1). Two assays quantify cell

viability/proliferation and three assays quantify cytotoxicity/apoptosis.

Assay 1 — Resazurin / Alamar Blue (Cell Viability)

Readout: % Viability vs Vehicle; normalization = $100 \times (\text{Sample} - \text{Blank}) / (\text{Vehicle} - \text{Blank})$. Higher % indicates more viable cells.

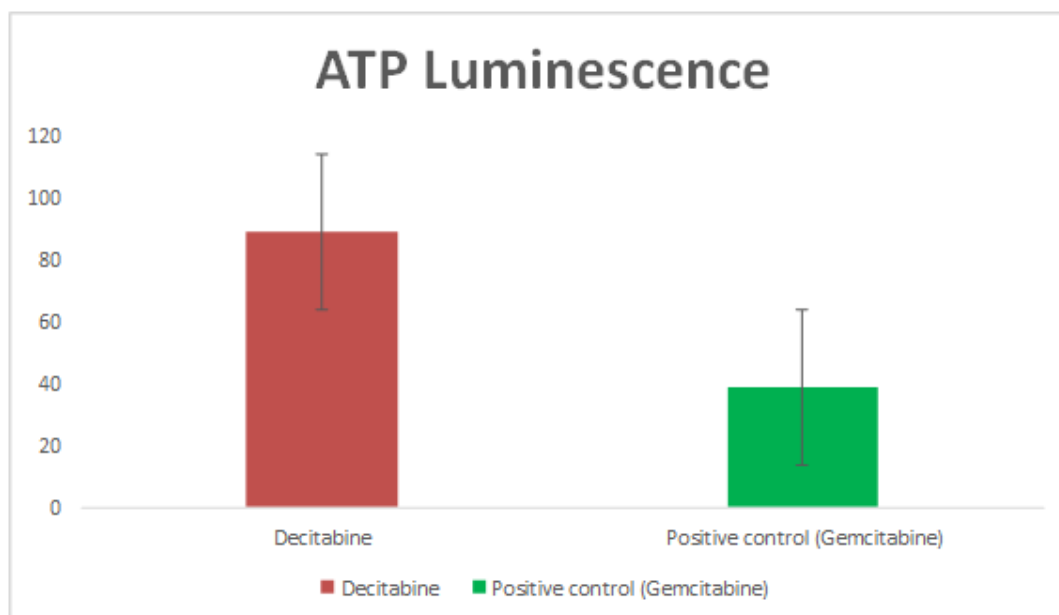
Group	Description	% Viability (vs Vehicle)	SD	n
G1	Decitabine	86	5	3
G2	Positive control (Gemcitabine)	44	5	3



Assay 2 — ATP Luminescence (Cell Viability)

Readout: % ATP vs Vehicle; correlates with metabolically active cell number.

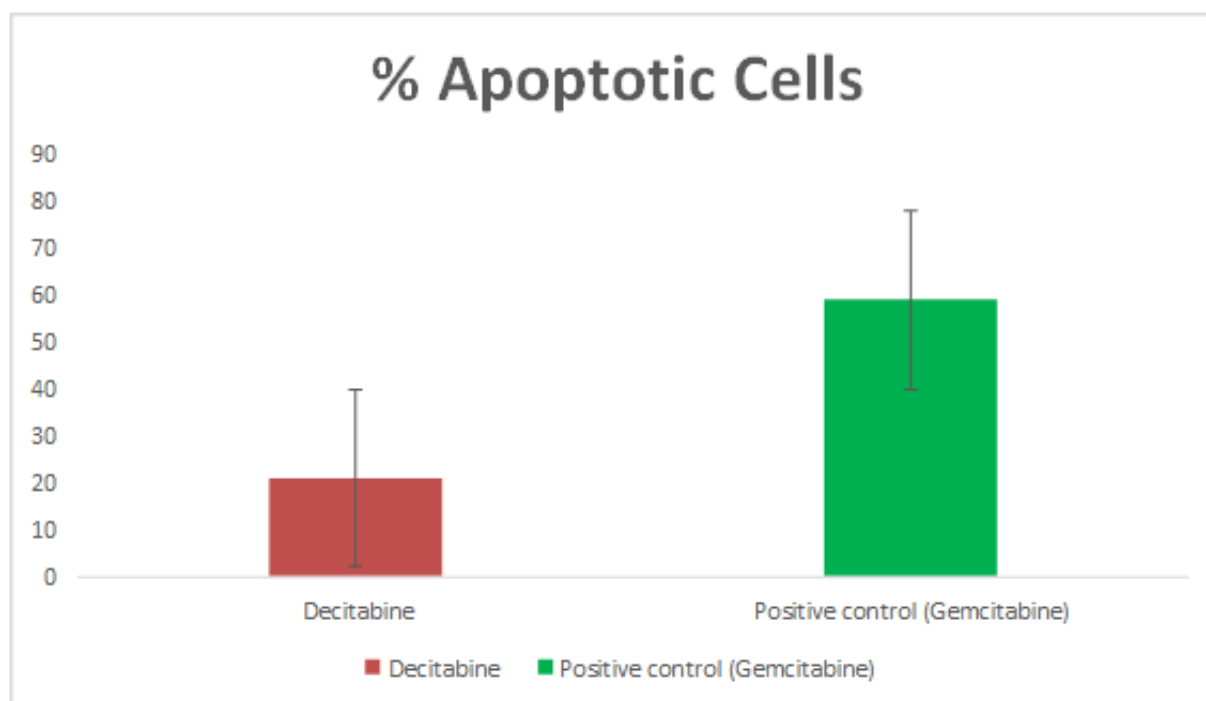
Group	Description	% ATP (vs Vehicle)	SD	n
G1	Decitabine	89	6	3
G2	Positive control (Gemcitabine)	39	5	3



Assay 3 — Annexin V / PI (Cytotoxicity)

Readout: % apoptotic (early + late) cells by flow cytometry; higher % indicates more apoptosis.

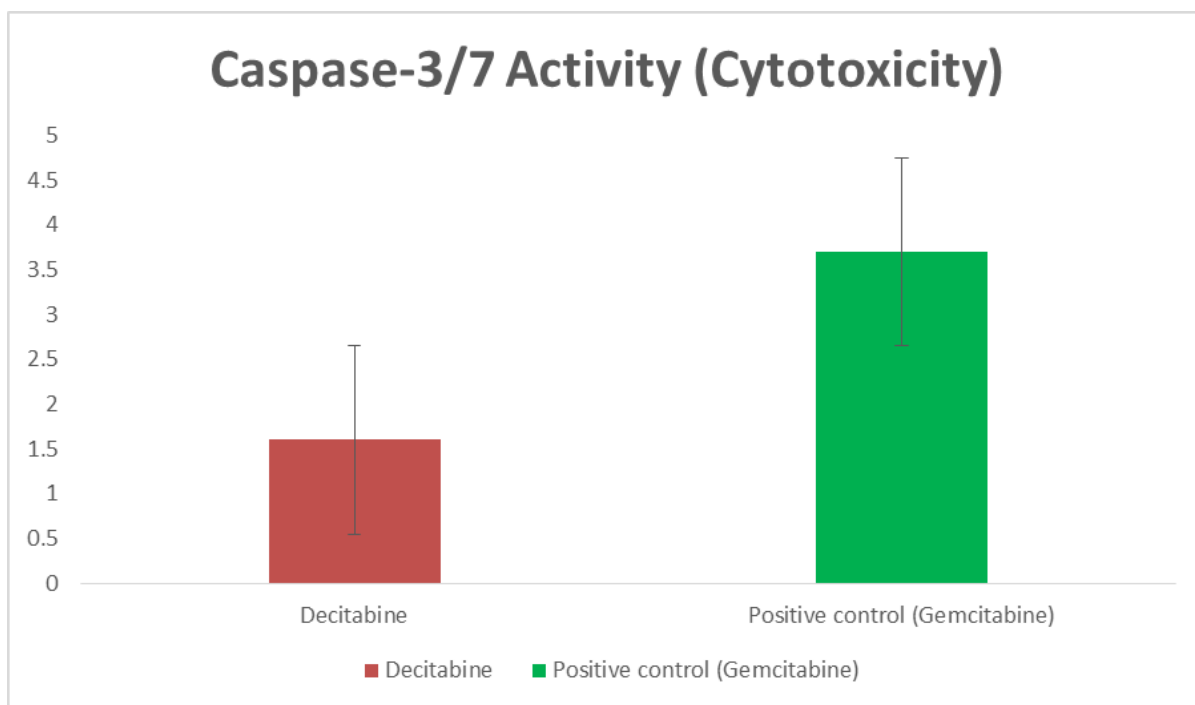
Group	Description	% Apoptotic Cells	SD	n
G1	Decitabine	21	3	3
G2	Positive control (Gemcitabine)	59	6	3



Assay 4 — Caspase-3/7 Activity (Cytotoxicity)

Readout: Fold-change in caspase-3/7 activity vs vehicle; executioner caspase activation during apoptosis.

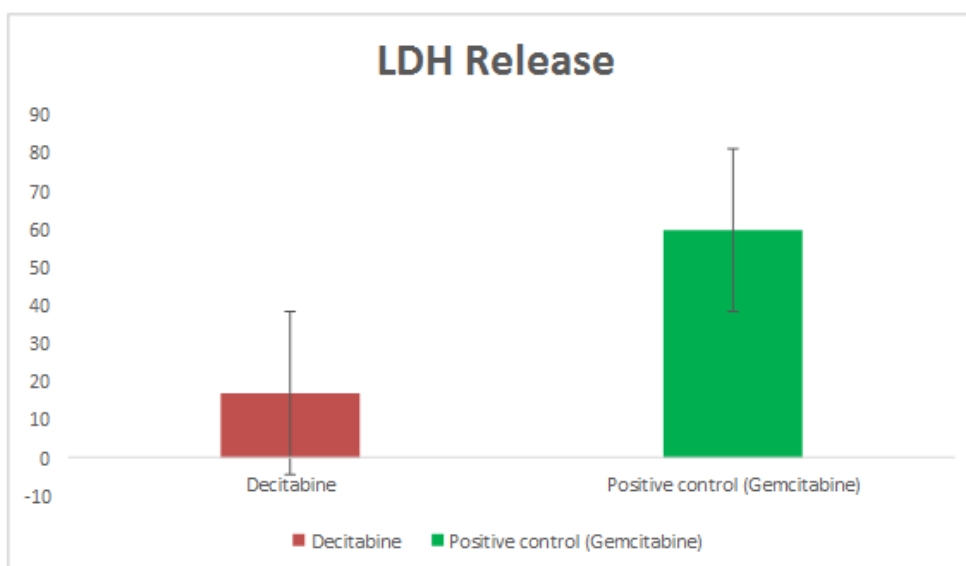
Group	Description	Fold-Change vs Vehicle	SD	n
G1	Decitabine	1.6	0.2	3
G2	Positive control (Gemcitabine)	3.7	0.3	3



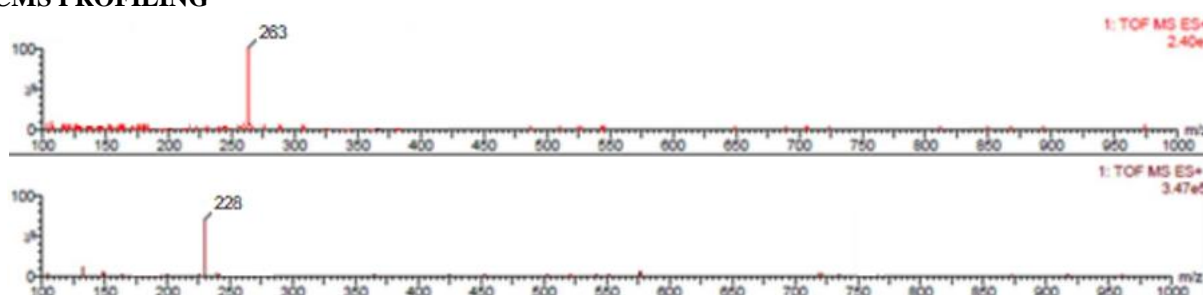
Assay 5 — LDH Release (Cytotoxicity)

Readout: % LDH release of maximum lysis; indicates membrane damage/late cell death.

Group	Description	% LDH Release (of Max)	SD	n
G1	Decitabine	17	3	3
G2	Positive control (Gemcitabine)	60	7	3



LCMS PROFILING



DISCUSSION

Decitabine exhibited weak antiproliferative and moderate apoptotic activity relative to *Gemcitabine*. Its viability scores above 85% indicate limited impact on cell proliferation, while modest caspase activation (1.6-fold) and low LDH release (17%) reflect **minimal cytotoxicity**. The observed 21% apoptotic fraction suggests that *Decitabine* induces partial programmed cell death, potentially through DNA demethylation-mediated gene reactivation rather than direct DNA damage. In contrast, *Gemcitabine* triggered extensive apoptosis and membrane rupture, validating its strong cytotoxic mechanism. The data underscore that *Decitabine* operates primarily as an **epigenetic modulator** rather than a classic cytotoxic agent. Thus, while its standalone efficacy in pancreatic cancer appears limited, it may sensitize resistant tumor cells when combined with nucleoside analogs or immune checkpoint therapies.

CONCLUSION

Decitabine demonstrates **modest antiproliferative and apoptotic effects** in pancreatic cancer cells with minimal cytotoxicity compared to *Gemcitabine*. Its favorable safety and mild apoptotic induction suggest potential as an adjunct in epigenetic combination therapies rather than as a primary cytotoxic drug. These results highlight the need for further studies exploring *Decitabine*'s synergistic potential and optimal dosing strategies in pancreatic cancer treatment paradigms.

BIBLIOGRAPHY

- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer statistics, 2008. *CA Cancer J Clin.*, 2008 Mar–Apr; 58(2): 71–96.
- ACS. *Cancer Facts & Figures 2008*. Atlanta: American Cancer Society; 2008.
- Carpelan-Holmstrom M, Nordling S, Pukkala E, Sankila R, Luttges J, Kloppel G, et al. Does anyone survive pancreatic ductal adenocarcinoma? A nationwide study re-evaluating the data of the Finnish Cancer Registry. *Gut.*, 2005 Mar; 54(3): 385–7.
- Jamieson JD. Prospectives for cell and organ culture systems in the study of pancreatic carcinoma. *J Surg Oncol.*, 1975; 7(2): 139–41.
- Longnecker DS, Wiebkin P, Schaeffer BK, Roebuck BD. Experimental carcinogenesis in the pancreas. *Int Rev Exp Pathol.*, 1984; 26: 177–229.
- Hall PA, Lemoine NR. Rapid acinar to ductal transdifferentiation in cultured human exocrine pancreas. *J Pathol.*, 1992 Feb; 166(2): 97–103.
- Habbe N, Shi G, Meguid RA, Fendrich V, Esni F, Chen H, et al. Spontaneous induction of murine pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of oncogenic Kras in adult mice. *Proc Natl Acad Sci U S A.*, 2008 Dec 2; 105(48): 18913–8.
- Park SW, Davison JM, Rhee J, Hruban RH, Maitra A, Leach SD. Oncogenic KRAS induces progenitor cell expansion and malignant transformation in zebrafish exocrine pancreas. *Gastroenterology.*, 2008 Jun; 134(7): 2080–90.
- Murtaugh LC, Leach SD. A case of mistaken identity? Noductal origins of pancreatic “ductal” cancers. *Cancer Cell.*, 2007 Mar; 11(3): 211–3.
- Githens S. Pancreatic duct cell cultures. *Annu Rev Physiol.*, 1994; 56: 419–43.
- Githens S. The pancreatic duct cell: proliferative capabilities, specific characteristics, metaplasia, isolation, and culture. *J Pediatr Gastroenterol Nutr.*, 1988 Jul–Aug; 7(4): 486–506.
- Bonner-Weir S, Toschi E, Inada A, Reitz P, Fonseca SY, Aye T, et al. The pancreatic ductal epithelium serves as a potential pool of progenitor cells. *Pediatric diabetes.*, 2004; 5 Suppl C: 16–22.
- Jones RT, Barrett LA, van Haaften C, Harris CC, Trump BF. Carcinogenesis in the pancreas. I. Long-term explant culture of human and bovine pancreatic ducts. *J Natl Cancer Inst.*, 1977 Mar; 58(3): 557–65.
- Oda D, Savard CE, Nguyen TD, Swenson ER, Lee SP. Culture of human main pancreatic duct epithelial cells. *In Vitro Cell Dev Biol Anim.*, 1998 Mar; 34(3): 211–6.
- Trautmann B, Schlitt HJ, Hahn EG, Lohr M. Isolation, culture, and characterization of human pancreatic duct cells. *Pancreas.*, 1993 Mar; 8(2): 248–54.
- Furukawa T, Duguid WP, Rosenberg L, Viallet J, Galloway DA, Tsao MS. Long-term culture and immortalization of epithelial cells from normal adult human pancreatic ducts transfected by the E6E7 gene of human papilloma virus 16. *Am J Pathol.*, 1996 Jun; 148(6): 1763–70.
- Ouyang H, Mou L, Luk C, Liu N, Karaskova J, Squire J, et al. Immortal human pancreatic duct epithelial cell lines with near normal genotype and phenotype. *Am J Pathol.*, 2000 Nov; 157(5): 1623–31.
- Lee KM, Yasuda H, Hollingsworth MA, Ouellette MM. Notch 2-positive progenitors with the intrinsic ability to give rise to pancreatic ductal cells. *Lab Invest.*, 2005 Aug; 85(8): 1003–12.
- Qian J, Niu J, Li M, Chiao PJ, Tsao MS. In vitro modeling of human pancreatic duct epithelial cell transformation defines gene expression changes induced by K-ras oncogenic activation in pancreatic carcinogenesis. *Cancer Res.*, 2005 Jun 15; 65(12): 5045–53.
- Campbell PM, Lee KM, Ouellette MM, Kim HJ, Groehler AL, Khazak V, et al. Ras-driven transformation of human nestin-positive pancreatic epithelial cells. *Methods Enzymol.*, 2008; 439: 451–65.
- Bendayan M, Duhr MA, Gingras D. Studies on pancreatic acinar cells in tissue culture: basal lamina (basement membrane matrix promotes three-dimensional reorganization. *Eur J Cell Biol.*, 1986 Oct; 42(1): 60–7.
- Longnecker DS, Lilja HS, French J, Kuhlmann E, Noll W. Transplantation of azaserine-induced

- carcinomas of pancreas in rats. *Cancer Lett.*, 1979 Aug; 7(4): 197–202.
23. Ulrich AB, Schmied BM, Standop J, Schneider MB, Pour PM. Pancreatic cell lines: a review. *Pancreas.*, 2002 Mar; 24(2): 111–20.
 24. Esni F, Miyamoto Y, Leach SD, Ghosh B. Primary explant cultures of adult and embryonic pancreas. *Methods Mol Med.*, 2005; 103: 259–71.
 25. Hober C, Benhamou PY, Watt PC, Watanabe Y, Nomura Y, Stein E, et al. A new culture method for human pancreatic islets using a biopore membrane insert. *Pancreas.*, 1997 Mar; 14(2): 199–204.
 26. Kenmochi T, Miyamoto M, Une S, Nakagawa Y, Moldovan S, Navarro RA, et al. Improved quality and yield of islets isolated from human pancreata using a two-step digestion method. *Pancreas.*, 2000 Mar; 20(2): 184–90.
 27. Lucas-Clerc C, Massart C, Campion JP, Launois B, Nicol M. Long-term culture of human pancreatic islets in an extracellular matrix: morphological and metabolic effects. *Molecular and cellular endocrinology.*, 1993 Jul; 94(1): 9–20.
 28. Yuan S, Rosenberg L, Paraskevas S, Agapitos D, Duguid WP. Transdifferentiation of human islets to pancreatic ductal cells in collagen matrix culture. *Differentiation.*, 1996 Oct; 61(1): 67–75.
 29. Beattie GM, Itkin-Ansari P, Cirulli V, Leibowitz G, Lopez AD, Bossie S, et al. Sustained proliferation of PDX-1+ cells derived from human islets. *Diabetes.*, 1999 May; 48(5): 1013–9.
 30. Lu J, Gu YP, Xu X, Liu ML, Xie P, Song HP. Adult islets cultured in collagen gel transdifferentiate into duct-like cells. *World J Gastroenterol.*, 2005 Jun 14; 11(22): 3426–30.
 31. Murray HE, Paget MB, Bailey CJ, Downing R. Sustained insulin secretory response in human islets co-cultured with pancreatic duct-derived epithelial cells within a rotational cell culture system. *Diabetologia.*, 2009 Jan 8.
 32. Mueller BM, Reisfeld RA. Potential of the scid mouse as a host for human tumors. *Cancer Metastasis Rev.*, 1991 Oct; 10(3): 193–200.
 33. Pantelouris EM. Absence of thymus in a mouse mutant. *Nature.*, 1968 Jan 27; 217(5126): 370–1.
 34. van Weerden WM, Romijn JC. Use of nude mouse xenograft models in prostate cancer research. *Prostate.*, 2000 Jun 1; 43(4): 263–71.
 35. Ikeda Y, Ezaki M, Hayashi I, Yasuda D, Nakayama K, Kono A. Establishment and characterization of human pancreatic cancer cell lines in tissue culture and in nude mice. *Jpn J Cancer Res.*, 1990 Oct; 81(10): 987–93.
 36. Rubio-Viqueira B, Jimeno A, Cusatis G, Zhang X, Iacobuzio-Donahue C, Karikari C, et al. An in vivo platform for translational drug development in pancreatic cancer. *Clin Cancer Res.*, 2006 Aug 1; 12(15): 4652–61.
 37. Walter K, Eshleman J, Goggins M. Xenografting and harvesting human ductal pancreatic adenocarcinomas for DNA analysis. *Methods Mol Med.*, 2005; 103: 103–11.
 38. Pretlow TG, Delmoro CM, Dilley GG, Spadafora CG, Pretlow TP. Transplantation of human prostatic carcinoma into nude mice in Matrigel. *Cancer Res.*, 1991 Jul 15; 51(14): 3814–7.
 39. Elsasser HP, Lehr U, Agricola B, Kern HF. Establishment and characterisation of two cell lines with different grade of differentiation derived from one primary human pancreatic adenocarcinoma. *Virchows Arch B Cell Pathol Incl Mol Pathol.*, 1992; 61(5): 295–306.
 40. Kobari M, Hisano H, Matsuno S, Sato T, Kan M, Tachibana T. Establishment of six human pancreatic cancer cell lines and their sensitivities to anti-tumor drugs. *Tohoku J Exp Med.*, 1986 Nov; 150(3): 231–48.
 41. Rasheed, A.; Farhat, R. Combinatorial Chemistry: A Review. *Int. J. Res. Pharm. Sci.*, 2013; 4: 2502–2516.
 42. Anas Rasheed*, Osman Ahmed. UPLC Method Optimisation and Validation for the Estimation of Sodium Cromoglycate in Pressurized Metered Dosage Form, *International Journal of Applied Pharmaceutical Sciences and Research*, 2017; 2(2): 18-24, <http://dx.doi.org/10.21477/ijapsr.v2i2.7774>
 43. Anas Rasheed*, Osman Ahmed. UPLC Method Development and Validation for the Determination of Chlophedianol Hydrochloride in Syrup Dosage Form. *International Journal of Applied Pharmaceutical Sciences and Research*, 2017; 2 (2): 25-31. <http://dx.doi.org/10.21477/ijapsr.v2i2.7775>
 44. Anas Rasheed*, Osman Ahmed. Validation of a Forced Degradation UPLC Method for Estimation of Beclomethasone Dipropionate in Respules Dosage Form. *Indo American Journal of Pharmaceutical Research*, 2017; 7(05).
 45. Anas Rasheed*, Osman Ahmed. Validation of a UPLC method with diode array detection for the determination of Noscapine in syrup dosage form. *European Journal of Pharmaceutical and Medical Research*, 2017; 4(6): 510-514.
 46. Anas Rasheed*, Osman Ahmed. Stability indicating UPLC method optimisation and validation of Triamcinolone in syrup dosage form. *World Journal of Pharmaceutical and Life Sciences*, 2017; 3, 4: 200-205.
 47. Anas Rasheed*, Osman Ahmed. Stability indicating UPLC method optimisation and validation of Pholcodine in bulk dosage form. *European Journal of Biomedical and Pharmaceutical Sciences*, 2017; 4, 6: 572-579.
 48. Anas Rasheed*, Osman Ahmed. Analytical method development and validation for the determination of Codeine in syrup dosage form using UPLC technology. *World Journal of Pharmaceutical and Life Sciences*, 2017; 3, 5: 141-145.
 49. Anas Rasheed*, Osman Ahmed. Analytical stability indicating UPLC assay and validation of Fluticasone propionate in nasal spray inhaler dosage form. *World*

- Journal of Pharmaceutical and Life Sciences, 2017; 3, 5: 168-172.
50. Anas Rasheed*, Osman Ahmed. Stability indicating UPLC method optimisation and validation of Acetylcysteine in syrup dosage form. *European Journal of Pharmaceutical and Medical Research*, 2017; 4(7): 485-491.
 51. Anas Rasheed*, Osman Ahmed. Analytical stability indicating UPLC assay and validation of Ciclesonide in dry powder inhaler dosage form. *European Journal of Pharmaceutical and Medical Research*, 2017; 4(7): 523-529.
 52. Anas Rasheed*, Osman Ahmed. Analytical stability indicating UPLC assay and validation of Dextromethorphan in syrup dosage form. *European Journal of Pharmaceutical and Medical Research*, 2017; 4(7): 548-554.
 53. Anas Rasheed*, Osman Ahmed. Analytical Development and Validation of a Stability Indicating Method for the Estimation of Impurities in Budesonide Respules Formulation, *International Journal of Applied Pharmaceutical Sciences and Research*, 2017; 2(3): 46-54. <http://dx.doi.org/10.21477/ijapsr.v2i3.8100>
 54. Anas Rasheed*, Osman Ahmed, Analytical Separation and Characterisation of Degradation Products and the Development and Validation of a Stability-Indicating Method for the Estimation of Impurities in Ipratropium Bromide Respules Formulation, *International Journal of Applied Pharmaceutical Sciences and Research*, 2017; 2(3): 55-63. <http://dx.doi.org/10.21477/ijapsr.v2i3.8101>