

How to Cite:

Hassan, S. S., Mukhtar, E. J. H. A., & Madlum, K. N. (2022). Metabolic pathways inhibitors can affect the therapeutic efficiency of doxorubicin. *International Journal of Health Sciences*, 6(S2), 9099–9108. <https://doi.org/10.53730/ijhs.v6nS2.7375>

Metabolic pathways inhibitors can affect the therapeutic efficiency of doxorubicin

Sara S. Hassan

Dept. of pharmacology and toxicology, college of medicine, University of Babylon, Iraq

Entisar J. Hamad Al Mukhtar

Dept. of pharmacology and toxicology, college of medicine, University of Babylon, Iraq

Kaiser N. Madlum

Dept. of human anatomy, college of medicine, University of Babylon, Iraq
Corresponding author email: kaiser.madlum@uobabylon.edu.iq

Abstract---Cancers are a group of diseases characterized by uncontrolled growth and the spread of abnormal cells. Colon cancer (CC) is one of the most aggressive tumor that leading to death in the world In this study, an attempt was made to evaluate the effect of different combinations of 2-deoxy-d-glucose, metformin, and doxorubicin on colon cancer cells viability. Results revealed a significant decrease in colon cancer viability after the treatment with combinations of (2-DG- metformin, 2-DG – Dox, and 2-DG- metformin - Dox) at certain concentrations.

Keywords---colon cancer, SW480, 2-deoxy-d-glucose, metformin, doxorubicin.

Introduction

Colon cancer (CC) is one of the leading death tumors in the world and it is considered among the big killers, together with lung, prostate, and breast cancer [1] and it befalls when there is an unrestrained cell proliferation in the large intestine and are usually originates from the noncancerous polyp which forms on the mucous membrane of the large intestine [2]. Traditional therapies for colon cancer include surgery followed by chemotherapy, targeted therapy, and immune therapy. Chemotherapeutic agents cause many side effects including digestive problems, leukopenia, and hair loss due to affecting normal cells through damaging DNA of the cell [3]. An alternative method was proposed to inhibit cancer growth via metabolic pathway blocking. the glucose metabolism inhibitor 2-DG

has a pronounced growth-inhibitory effect on cancer cells both in vitro and in vivo especially inhibition of glycolysis, and only a few studies addressed the question of altered glycosylation or other metabolism-independent effects of 2-DG [4].

Metformin is an anti-diabetic drug that now is drawing much attention as an anti-tumor agent, it inhibits cell proliferation by inducing cell cycle arrest and has ability to the re-expression of specific genes [5]. It can enhance proliferation and attachment of colon cancer at lower concentrations [6]. Metformin can also decrease the incidence of carcinomas, improve response of cancer cells to management with chemotherapy and radiotherapy, reduce metastasis and reduce the relapse. Therefore, this agent can be used as a both for the treatment and prevention of cancer [7] [8]. Doxorubicin (DOX) is a widely used anthracycline-based antitumor agent for both solid and liquid tumors. DOX, are designed to kill cancer cells by generating oxidative stress. However, the administration of DOX frequently results in the development of drug resistance, a critical hurdle in cancer treatment [9]. The primary mechanism of action of DOX involves the drug's ability to intercalate within DNA base pairs, causing breakage of DNA strands and inhibition of both DNA and RNA synthesis. DOX inhibits the enzyme topoisomerase II, causing DNA damage and induction of apoptosis [10]. In this study, we tried to target both inhibition of glycolysis (by using 2-DG) and Oxidative phosphorylation (using Metformin) pathways to inhibit cancer energy sources essential for cells growth, proliferation, and invasion.

Materials and Methods

Colon cancer cell line

A human colon cancer cell line (SW480) (kindly provided by the cancer research laboratory at Babylon university) was used in this study. Cells were propagated in a monolayer using (RPMI-1640) medium supplemented with 10% fetal bovine serum (FBS) and 1% gentamicin.

Anticancer agents

Metformin, 2-deoxy-D-glucose (Merk) and Doxorubicin (Pfizer) were diluted in RPMI-1640 (Capricorn) to obtain the stock solutions used to treat the cells.

Cytotoxicity assay

Cells were seeded in 96-tissue culture treated well plates and incubated for 24 hours, then media were removed and a new media containing experimental drugs at desired concentrations. Plates were incubated again for 24 hours, then for each well, 200 μ l of MTT solution (5 milligram per ml) was added and incubated at 37°C for 4 hours. After that remove media, and then DMSO (200 μ l) added to all wells. Optical density was read at 570 nm on a microtiter scanning plate reader (Human)[11].

Statistical analysis

Data were analyzed using Microsoft Office Excel 2019 and Sigmaplot v 12.5. Differences between mean concentrations tested were analyzed using the one-way Anova test. The P-value ≤ 0.05 and ≤ 0.001 was considered statistically significant and highly significant, respectively.

Results

Cytotoxicity of 2-DG on SW480 colon cancer

Results showed that 2-DG at concentrations from (500 to 4000 $\mu\text{g/ml}$) causes a significant inhibition in the viability of colon cancer cells as shown in figure 1.

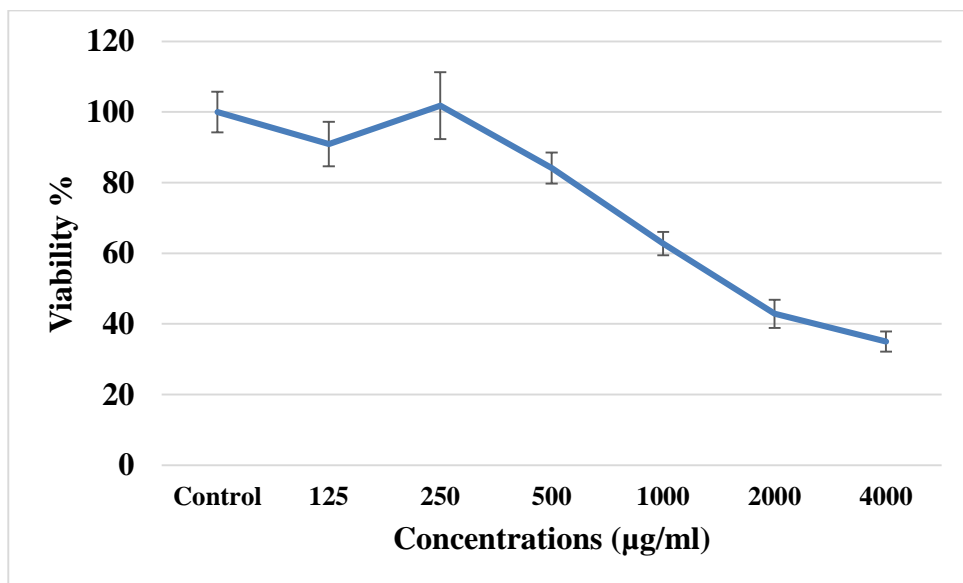


Figure 1: Dose dependant cytotoxicity of 2-DG on SW480 cells.

Cytotoxicity of Metformin on SW480 colon cancer

The results revealed in figure 2 revealed that metformin at the high concentrations (4000, and 2000 $\mu\text{g/ml}$) causes a highly significant decrease in cells viability while lower concentrations didn't affect cells viability.

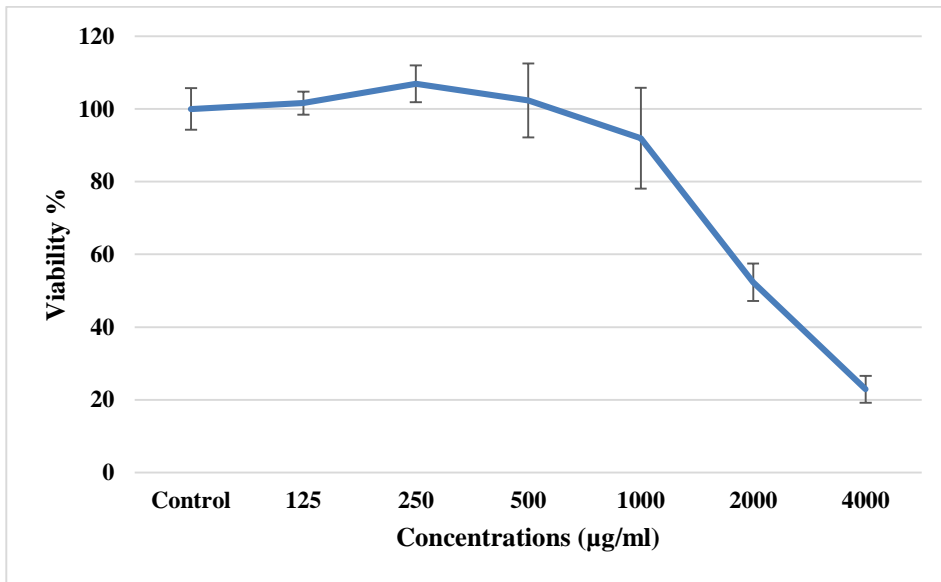


Figure 2: Dose dependant cytotoxicity of Metformin on SW480 cells

Cytotoxicity of DOX on SW480 colon cancer

Results showed that DOX at all concentrations used (from 250 - 7.8 µg/ml) causes a significant ($P < 0.050$) decrease in the viability of SW480 colon cancer cells compared to the control group as shown in figure 3.

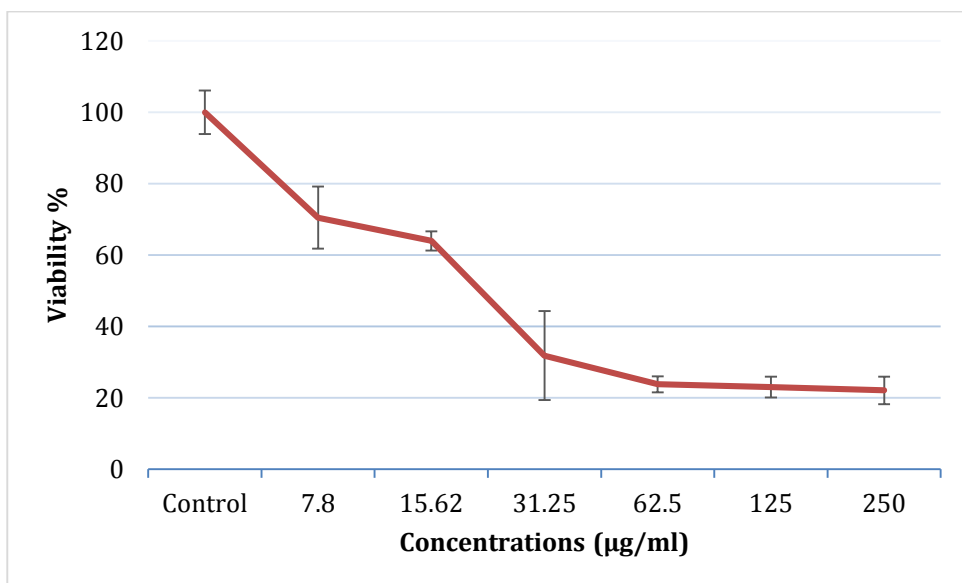


Figure 3: Effect of DOX on colon cancer cells viability

Cytotoxicity of (2-DG – metformin) combination on SW480 colon cancer

The administration of (2-DG with metformin) combinations at different concentrations on the cells significantly ($p < 0.05$) inhibits cell growth at all concentrations, The most effective combination was that containing (2-DG 1000 $\mu\text{g}/\text{ml}$ + metformin 4000 $\mu\text{g}/\text{ml}$) as shown in figure 4.

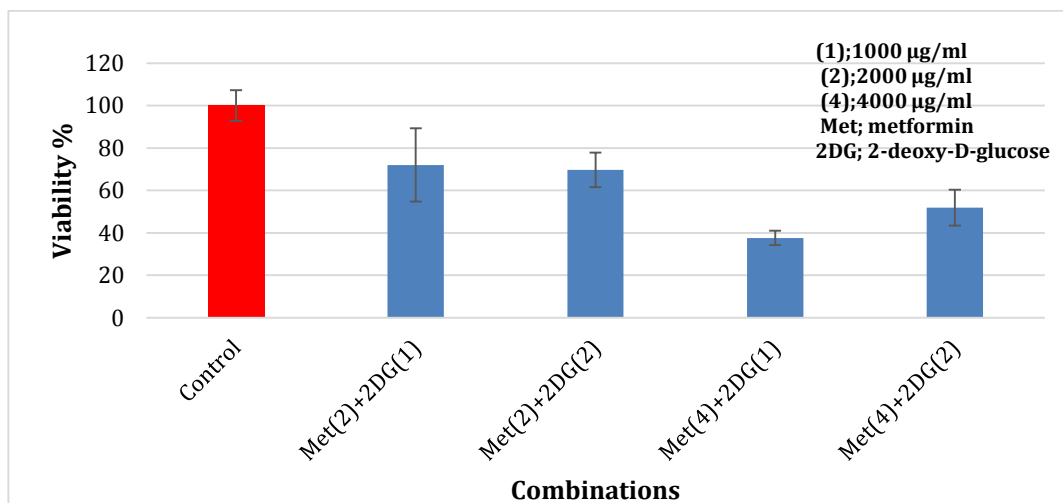


Figure 4: Effect of 2-DG -Metformin combination on colon cancer cells viability

Effect of (2-DG – metformin) combination on doxorubicin cytotoxicity

As shown in figure 5, the co-administration of 2-DG or Metformin with doxorubicin has a great influence on its cytotoxicity. The administration of both (2-DG and metformin) with doxorubicin significantly decrease cell viability compared to the effect of this drug alone or combined with only one of these chemicals.

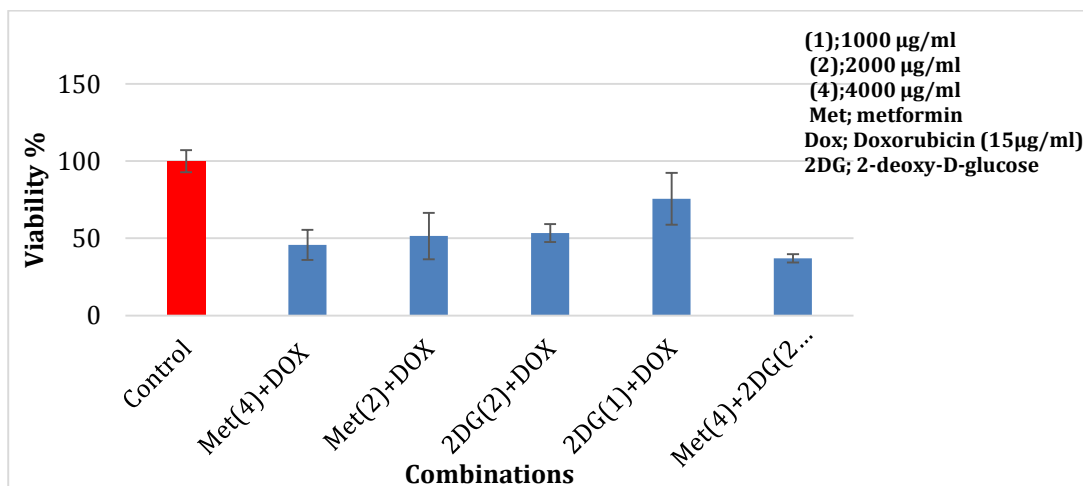


Figure 5: Effect of Doxorubicin, 2-DG and metformin combinations on SW480 colon cancer cells viability

Discussion

Our results revealed that there was a significant decrease in colon cancer SW480 cell viability at the high concentrations of 2-DG, while low concentrations have no effect on cell viability in comparing to control group as shown in figure 1, this effect may be related to glucose deprivation of cancer cell as have been reported by many studies which have confirmed that the 2-DG has a noticeable growth-inhibitory effect on cancer cells [12]. In addition that 2DG inhibits glycolysis due to the formation and intracellular accumulation of 2-deoxy-d-glucose-6-phosphate (2-DG6P), inhibiting the function of hexokinase and glucose-6-phosphate isomerase, and inducing cell death [13]. 2-DG also have ability to alter glycosylation. However, an effect of 2-DG on N-linked glycosylation seems to play an important role in mediating toxicity in tumor cells under certain conditions [14]. A study using HeLa cells shows that the glucose metabolism inhibited by 2-DG, is cytotoxic and have the ability to enhance radiosensitization in human cancer cells in vitro [15].

Metformin, an oral antidiabetic drug, is being evaluated in multiple clinical trials as an adjuvant drug to chemotherapy. Especially, after several groups of researchers approved its activity as anti-cancer by increasing apoptosis [16]. The result of this work revealed that metformin significantly inhibited SW480 colon cell proliferation at higher concentrations (2000, 4000 µg/ml).

Studies by Yang *et al* and Coronel-herna *et al* documented similar findings. They established that metformin cause inhibition in cell proliferation by causing cell cycle arrest in different cell line models of kidney, breast, pancreatic, prostate and colon cancers [17] [18]. Other study achieved by Lesan *et al* revealed that metformin arrested cell cycle at phase G0/G1, resulting in accumulation of cell population at this phase which in turn led to induction of apoptosis [19]. Moreover, in vitro study demonstrated that Metformin may activate ERK/MAPK pathways. ERK is a signaling pathway that has been well documented to have a major role in regulating proliferation and apoptosis [20]. A clinical study found that patients use metformin in low doses for one year reduced the probability of colorectal cancers [21].

Doxorubicin (DOX) is a widely used anthracycline-based antitumor agent for both solid and liquid tumors. DOX, are designed to kill cancer cells by generating oxidative stress. [22] The result showed that all used concentrations of DOX (Form 250-7.8 µg/ml) causes a significant decrease in the viability of SW480 colon cancer cells compared to the control group as shown in (figure 3)

The DOX is a well-known chemotherapeutic agent which is used in the treatment of a wide variety of cancers inducing intracellular ROS accumulation, cell cycle arrest, and apoptosis [23]. Also a study by Lüpertz *et al.*, (2010) investigate the effects of DOX in Hct-116 human colon carcinoma cells to clarify if a time/concentration range for optimal DOX-induced apoptosis exists. found that bolus treatment with DOX resulted in a dose-dependent decrease of viable cells and concomitant increase of apoptosis, In contrast, continuous treatment with DOX reduced the number of living cells with no parallel raise in the number of

dead cells ^[24]. DOX inhibited tumor growth in the SW480 CRC xenograft model and stopped cell cycle progression in CT-26 murine CRC cells ^[25].

The result of (figure 4) revealed that the combined effect of 2-DG and metformin significantly reduced cell viability compared to the effect of each one alone. The combinational use of low-dose 2-DG and Metformin can markedly inhibit cell proliferation via simultaneously inhibiting glycolysis and oxidative phosphorylation metabolism, reducing intracellular ATP production, activating AMPK activity, and thereby inhibiting the activation of the mTOR proliferation signaling pathway in kidney epithelial cells ^[26]. Combined treatment with metformin and 2-DG did not increase the percentage of dead PC-3 cells; however, it also did not induce their detachment ^[27].

Because DOX is toxic and causes serious side effects in cancer patients ^[28] a major challenge is to lower its side effects without decreasing its effectiveness. Results showed that the growth of SW480 colon cancer cells was significantly inhibited after the treatment with DOX in combination with 2-DG or metformin or both, the most effective combination was that containing (DOX + metformin + 2-DG) as shown in (figure 5).

The 2-DG is efficient to inhibit the glycolysis of cancer cells and block the intratumoral energy supply, which works in synergy with the co-loaded chemotherapeutic drug Dox to promote superoxide anion generation and mitochondrial depolarization, finally leading cancer cells to pro-apoptotic pathways. Comparatively, the starvation effect of 2-DG can neutralize the toxicity of Dox in normal cells, thus mitigating the side effect of chemotherapy ^[29].

A recent study observed that 2-DG enhances the effects of two agents which are known to act on DNA, DOX, and 5FU. Doxorubicin, a member of the anthracycline family, is known to cause the generation of intracellular superoxide and hydrogen peroxide, which can mediate mitochondrial damage and apoptosis in a p. 53-independent manner. The study also found that 2-DG treatment results in increased production of reactive oxygen species ^[30]. Another study also observed that induction of autophagy by combination therapy had an anti-tumoral role, suggesting that the success of therapy is due to repressing DNA replication by DOX and targeting aberrant metabolism with metformin. It has been reported that metformin and DOX arrest cell cycle and induce cell death through negative regulation of Phosphatidylinositol-3-kinase (PI3K) PI3K/AKT pathway ^[31]. Metformin and DOX mono-treatments exhibited opposing action regarding phosphorylated adenosine monophosphate protein kinase. Co-treatment markedly decreased tumor volume, increased survival rate, and improved other parameters compared to DOX treatment alone ^[32].

Conclusions

The high concentration of 2deoxy-d-glucose, Metformin had a greater cytotoxic effect on SW480 colon cancer. These two drugs can be used as an effective anticancer adjuvant, alone or together to enhance DOX toxicity. The benefits of these combinations are to lowering the chemotherapy drugs doses and time of administration, leading to reducing their side effects on the patients.

References

1. Labianca, Roberto, Beretta, G. D., Kildani, B., Milesi, L., Merlin, F., Mosconi, S., Pessi, M. A., Prochilo, T., Quadri, A., Gatta, G., de Braud, F., & Wils, J. (2010). Colon cancer. *Critical Reviews in Oncology/Hematology*, 74(2), 106–133. <https://doi.org/10.1016/j.critrevonc.2010.01.010>
2. Rajalekshmi M, Shreedhara CS, Lobo R, Rao PP. The review on genetics, epigenetics, risk factors and diagnosis of colon cancer. Vol. 11, *Research Journal of Pharmacy and Technology*. 2018.
3. Munker S, Gerken M, Fest P, Ott C, Schnoy E, Fichtner-Feigl S, et al. Chemotherapy for metastatic colon cancer: No effect on survival when the dose is reduced due to side effects. *BMC Cancer*. BMC Cancer; 2018;18:1–9.
4. Kurtoglu, M., Gao, N., Shang, J., Maher, J. C., Lehrman, M. A., et al. (2007) Under normoxia, 2-deoxy-D-glucose elicits cell death in select tumor types not by inhibition of glycolysis but by interfering with N-linked glycosylation. *Mol. Cancer Ther.* 6, 3049–3058.
5. Joshi RS, Nangare AK, Sanap DS, Sase SM. Development and Validation of UV-Spectrophotometric Method for Estimation of Metformin Hydrochloride and Pioglitazone in Tablet Dosage Form. *J Drug Deliv Ther.* 2019;9(4-A).
6. Rashid AI, Madlum KN, Ghaleb R, Obied HN, Enayah SH. Effects of antidiabetic drug on the anticancer activity of Cisplatin © *Annals of Tropical Medicine & Public Health S280* © *Annals of Tropical Medicine & Public Health S280*. *Annals of Tropical Medicine and Public Health*. 2019;22.
7. . Saraei P, Asadi I, Kakar MA, Moradi-Kor N. The beneficial effects of metformin on cancer prevention and therapy: A comprehensive review of recent advances. *Cancer Management and Research*. 2019;11:3295–313.
8. El-ashmawy, N. E., Khedr, N. F., & El-bahrawy, H. A. (2017). Metformin augments doxorubicin cytotoxicity in mammary carcinoma through activation of adenosine monophosphate protein kinase pathway. <https://doi.org/10.1177/1010428317692235>.
9. Locatelli, L., Cazzaniga, A., Fedele, G., Zocchi, M., Scrimieri, R., Moscheni, C., Castiglioni, S., & Maier, J. A. (2021). A comparison of doxorubicin-resistant colon cancer lovo and leukemia hl60 cells: Common features, different underlying mechanisms. *Current Issues in Molecular Biology*, 43(1), 163–175. <https://doi.org/10.3390/cimb43010014>.
10. Johnson-arbor, K., & Dubey, R. (2020). Doxorubicin.
11. Johan van Meerloo, Gertjan J.L. Kaspers and JC. *Cancer Cell Culture*. *Cancer Cell Culture*. 2011;731:237–45.
12. Kurtoglu M, Maher JC, Lampidis TJ. Differential toxic mechanisms of 2-deoxy-D-glucose versus 2-fluorodeoxy-D-glucose in hypoxic and normoxic tumor cells. *Antioxidants and Redox Signaling*. 2007;9:1383–90.
13. Blum, R.; Kloog, Y. Metabolism addiction in pancreatic cancer. *Cell Death Dis*. 2014, 5, e1065.
14. Raez, L. E., Papadopoulos, K., Ricart, A. D., Chiorean, E. G., Dipaola, R. S., et al. (2013) A phase I dose-escalation trial of 2-deoxy-D-glucose alone or combined with docetaxel in patients with advanced solid tumors. *Cancer Chemother*.
15. Tuccillo FM, de Laurentiis A, Palmieri C, Fiume G, Bonelli P, Borrelli A, et al. Aberrant glycosylation as biomarker for cancer: Focus on CD43. *BioMed Research International*. Hindawi Publishing Corporation; 2014.

16. . Haffty, B. G. et al. Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. *J. Clin. Oncol.* 24, 5652–5657, doi:10.1200/JCO.2006.06.5664 (2006).
17. Saini N, Yang X. Metformin as an anti-cancer agent: Actions and mechanisms targeting cancer stem cells. *Acta Biochimica et Biophysica Sinica.* 2017;50:133–43.
18. Coronel-herna J, Cantu D, Jacobo-herrera N, Millan-catalan O, Delgado-waldo I, Campos-parra AD, et al. Combination of Metformin , Sodium Oxamate and Doxorubicin Induces Apoptosis and Autophagy in Colorectal Cancer Cells via Downregulation HIF-1 a. 2021;11:1–13.
19. Lesan, V., Ghaffari, S. H., Salaramoli, J., Heidari, M., Rostami, M., Alimoghaddam, K., & Ghavamzadeh, A. (2014). Evaluation of antagonistic effects of metformin with cisplatin in gastric cancer cells. *International Journal of Hematology-Oncology and Stem Cell Research*, 8(3), 12–19.
20. Higurashi T, Hosono K, Takahashi H, Komiya Y, Umezawa S, Sakai E, et al. Metformin for chemoprevention of metachronous colorectal adenoma or polyps in post-polypectomy patients without diabetes: a multicentre double-blind, placebo-controlled, randomised phase 3 trial. *The Lancet Oncology* [Internet]. Elsevier Ltd; 2016;17:475–83.
21. Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, et al. The antidiabetic drug metformin exerts an anti- tumoral effect in vitro and in vivo through a decrease of cyclin D1 level. *Oncogene* 2008;27:3576–86.
22. Locatelli, L., Cazzaniga, A., Fedele, G., Zocchi, M., Scrimieri, R., Moscheni, C., Castiglioni, S., & Maier, J. A. (2021). A comparison of doxorubicin-resistant colon cancer lovo and leukemia hl60 cells: Common features, different underlying mechanisms. *Current Issues in Molecular Biology*, 43(1), 163–175. <https://doi.org/10.3390/cimb43010014> .
23. Putri, H., Jenie, R. I., Handayani, S., Kastian, R. F., & Meiyanto, E. (2016). Combination of potassium pentagamavunon-0 and doxorubicin induces apoptosis and cell cycle arrest and inhibits metastasis in breast cancer cells. *Asian Pacific Journal of Cancer Prevention*, 17(5), 2683–2688.
24. Lüpertz, R., Wätjen, W., Kahl, R., & Chovolou, Y. (2010). Dose- and time-dependent effects of doxorubicin on cytotoxicity, cell cycle and 87 apoptotic cell death in human colon cancer cells. *Toxicology*, 271(3), 115–121. <https://doi.org/10.1016/j.tox.2010.03.012>
25. Emami RA, Fisel P, Nies AT, Schaeffeler E, Schwab M. Metformin and cancer: from the old medicine cabinet to pharmacological pitfalls and prospects. *Trends Pharmacol Sci* 2013;34:126–35.
26. Cheong JH, Park ES, Liang J, Dennison JB, Tsavachidou D, Nguyen-Charles C, et al. Dual inhibition of tumor energy pathway by 2-deoxyglucose and metformin is effective against a broad spectrum of preclinical cancer models. *Molecular Cancer Therapeutics*. 2011;10:2350–62.
27. Bizjak M, Malavašič P, Dolinar K, Pohar J, Pirkmajer S. Combined treatment with Metformin and 2-deoxy glucose induces detachment of viable MDA-MB-231 breast cancer cells in vitro. 2017;(October 2016):1–14.
28. Johnson-arbor K, Dubey R. Doxorubicin. 2020;
29. Yang, B., Chen, Y., & Shi, J. (2020). Tumor-Specific Chemotherapy by Nanomedicine-Enabled Differential Stress Sensitization. *Angewandte Chemie*, 132(24), 9780–9788.

30. Zhang, D., Li, J., Wang, F., Hu, J., Wang, S., & Sun, Y. (2014). 2- Deoxy-D-glucose targeting of glucose metabolism in cancer cells as a potential therapy. *Cancer Letters*, 355(2), 176–183.
31. Babichev Y, Kabaroff L, Datti A, Uehling D, Isaac M, Al-awar R, et al. Pi3k/Akt/mTOR Inhibition in Combination With Doxorubicin is an Effective Therapy for Leiomyosarcoma. *J Trans Med* (2016) 14:67. doi: 10.1186/s12967-016-0814-z.
32. El-Ashmawy NE, Khedr NF, El-Bahrawy HA, Abo Mansour HE. Metformin augments doxorubicin cytotoxicity in mammary carcinoma through activation of adenosine monophosphate protein kinase pathway. *Tumor Biology*. May 2017.