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Evaluation the Impact of Silibinin on governing the resistance to 5-fluruuracil in Colorectal cancer cell line (sw480) via adjusting the mTOR signaling pathway

Rusul Ali Kadhim [1] , Rana A. Ghaleb [2]

Abstract

Colorectal cancer (CRC) ranks as one of the most prevalent malignant tumors It is anticipated that by 2035, the overall number of fatalities from rectal and colon cancer will grow by 60% and 71.5%, respectively Silibinin, a polyphenol flavonoids, is the primary active component of siylmarin. It extract from dried Sylibum marianum (milk thistle) seeds. It has pleotropic role as anti-inflammatory, hepatoprotective and anticancer effect.

The study aimed to estimate the impact of silibinin on modulation the resistance in CRC cell line to 5- fluorouracil (5-FU) via alteration apoptotic markers and mTOR pathway genes.

The SW480 cell line was treated with different doses of silibinin monotherapy and in combination with 5-Flurouracil IC-50.- "MTT assay" used for cytotoxic assessment. qPCR used for . Level of expression of PI3K/AKT/mTOR genes . ELISA technique For apoptotic markers. -- microplate florescence reader used rhodamine123 dye measured the intracellular accumulation of rhodamine 123 . Results showed - high significant reduction in the growth of sw480 cancer cells for all concertation of silibinin as monotherapy and combination with 5-FU .- The combination of silibinin with 5-fu reveled highly significant $p<0.0005$ increased the retention of 123rheodamin within the sw480 cells when compare with untreated control group . Both concentrations of silibinin(15,500µm) when combined with 5-fu (300µm) achieved significant reduction of folding change for the PI3KCA,AKT and mTOR genes when compared with control untreated group.

Conclusion Silibinin may interfere and reduce the growth of colorectal cancer cells sw480 via modifying the genes that are over-regulated in cancer cells

Keywords: Silibinin, colorectal cancer, SW480, gene folding, mTOR pathway

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تقييم تأثير السيليبينين على التحكم في مقاومة 5-فلورويوراسيل في خط خلايا سرطان القولون والمستقيم (sw480) من خلال تعديل مسار إشاراتmTOR

رسل علي كاظم [1] ، رنا اياد غالب [2]

المستخلص

يُعد سرطان القولون والمستقيم (CRC) أحد أكثر الأورام الخبيثة انتشارًا. ومن المتوقع أن يرتفع إجمالي عدد الوفيات الناجمة عن سرطان المستقيم والقولون بنسبة 60% و71.5% على التوالي بحلول عام 2035. يُعد السيليبينين، وهو أحد فلافونويدات البوليفينول، المكون النشط الرئيسي في السيلمارين. يُستخرج السيليبينين من بذور نبات الشوك المجفف (Sylibum marianum). يتميز السيليبينين بتأثيره متعدد التأثيرات كمضاد للالتهابات، وواقي للكبد، ومضاد للسرطان.

هدفت الدراسة إلى تقدير تأثير السيليبينين على تعديل مقاومة خط خلايا سرطان القولون والمستقيم لـ 5-فلورويوراسيل (5-FU) من خلال تغيير علامات موت الخلايا المبرمج وجينات مسار mTOR.

عولجت سلالة خلايا SW480 بجرعات مختلفة من السيليبينين أحادي العلاج، وبالاشتراك مع 5-فلورويوراسيل IC-50. - "اختبار MTT" المستخدم لتقييم السمية الخلوية. استُخدم تفاعل البوليميراز المتسلسل الكمي (qPCR) لـ... مستوى التعبير عن جينات PI3K/AKT/mTOR. تقنية ELISA

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معلومات البحث
تأريخ النشر: تشرين الاول 2025

لعلامات موت الخلايا المبرمج. -- استخدم قارئ فلورسنت الصفيحة الدقيقة صبغة روداميين 123 لقياس تراكم روداميين 123 داخل الخلايا
- أظهرت النتائج انخفاضًا ملحوظًا في نمو خلايا سرطان sw480 لجميع حالات استخدام السيليبينين كعلاج وحيد أو مع 5-FU. أظهر الجمع بين السيليبينين و5-FU زيادة ملحوظة في احتباس rheodamin123 داخل خلايا sw480 عند مقارنتها بمجموعة التحكم غير المعالجة. حقق كلا تركيزي السيليبينين (15,500 ميكرومتر) عند دمجهما مع 5-300) FU ميكرومتر) انخفاضًا ملحوظًا في تغير الطي لجينات PI3KCA وAKT وmTOR عند مقارنتها بمجموعة التحكم غير المعالجة.
الاستنتاج
قد يتداخل السيليبينين مع نمو خلايا سرطان القولون والمستقيم ويقلله عن طريق تعديل الجينات المفرطة التنظيم في خلايا السرطان).

الكلمات المفتاحية: سيليبينين، سرطان القولون والمستقيم، SW480، طي الجينات، مسار mTOR -

Introduction

Colorectal cancer (CRC) is the third most prevalent cause of death in men and the fourth for women globally which is typified by abnormal cellular energetics [1]. It is a critical public health issue and it is linked to high rates of morbidity and mortality in the western region as well as in countries with similar dietary and lifestyle patterns [2]. It is the common outcome of a wide range of genetic (gene mutations, chromosomal abnormalities) and epigenetic modifications seen in normal colorectal cells [3]. MDR, caused by overexpression of P-glycoprotein (P-gp) in cancer tissues, is the primary reason for chemotherapy failure in colorectal cancer (CRC) patients. Despite research efforts to circumvent P-gp-mediated medication efflux, the high toxicity of P-gp inhibitors has been a major barrier to their clinical usage[4].

The phosphoinositide 3-kinase (PI3K) pathway is one of the most triggered pathways in cancer, and its activation is central to most altered metabolic pathways for promoting the anabolic processes needs of developing cancer cells [5]. Multiple research investigations have found aberrant PI3K/Akt/mTOR pathway regulation in various malignancies, including breast, liver, colorectal, prostate, and gastric cancer [6]. Optimal concentrations of free radicals are needed in different biological activities.

Overproduction of ROS causes imbalance and degeneration of cell components, including nucleic acids, proteins, and lipids, through interactions with macromolecules. [7]. These ROS have the ability to oxidize biological macromolecules, particularly nucleic acids, by producing protein-DNA cross-links, DNA strand breaks, single nucleobase lesions, and cross-linking of intra- and inter-strands of nucleic acid bases[8]. Inhibition of antiapoptotic molecules and activation of proapoptotic molecules are two popular approaches for therapeutic targeting [9]. Targets that have been studied include BCL-2 inhibitors [10], death-receptor ligands and alkylphospholipid analogues (APL), which function as apoptotic signals. Although treatment can be directed towards any point of the pathways, it is not certain which target will work best. The most effective targets will be identified as additional anticancer medications that induce apoptosis are developed[11]. Caspase cascades play critical functions in apoptosis and are strongly linked to cancer progression and prognosis. Low caspase expression or inactivation in cancer cells can make them resistant to microenvironmental tensions and therapies[12]. The seeds of Silybum marianum

(L.) Gaertn (family Asteraceae), also known as milk thistle, are the source of silibinin (C25H22O10). In a number of human malignant tumours, such as skin, bladder, prostate, and colon cancer, silibinin has been shown to exhibit pleiotropic anticancer activity. In addition to being a sensitiser, silibinin is also employed as a medicinal drug[13]. Silymarin has been shown to modulate cell cycle, prevent tumour development and spread, cause apoptosis, alter immunological responses to tumour cells, and decrease angiogenesis. Silymarin's tumoricidal properties stem from its modulatory effects on cell signalling pathways such as MAPK, PI3K/Akt, Wnt/β-Catenin, STAT3, and NFκB (14).

Material and methods

Chemicals and reagents

RPMI-1640 media and phosphate buffered saline PBS from Gibco/UK, foetal bovine serum from Capricorn/Germany, penicillin-streptomycin solution from Elsbscience/China, and gentamycin (80mg vial) from The Arab Pharma/Jordan. Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) dye powder (MTT) was procured from Roth/Germany, dimethylsulphoxide (DMSO) from (Bio Basic Inc/Canada), primers from (Macrogen/Korea), 5-flurouracil (50mg/ml) from (Onko ilac san/Turkey), fluorescence Rhodamin123 dye (98% purity) was acquired from Heonwns/China, 5gm of Silibinin (98% purity) from Sigma Aldrich/USA, Apoptotic markers caspaes-3 and BCL-2 provided from BT LAB China. qpcr kits total RNA extraction kit from Solarbi/China, and cDNA Synthesis Kit 22701/Addbio (Korea).

Cell culture: Colorectal Cancer "SW480" Cell Lines Sigma Aldrich provided frozen vials from a 50-year-old male's primary adenocarcinoma tumor. The cells were grown in RPMI-1640 media with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified 37° C incubator.

Stocks prepration:

In accordance with the guidelines provided by Sigma Alderch, 5 mg of silibinin was dissolved in 1 ml of DMSO using a vertex until a clear solution was achieved. The final DMSO concentration was not greater than 0.1% (v/v). A working solution was then made from this stock solution by diluting it with Roswell Park Memorial Institute (RPMI-1640). By diluting this stock vial with RPMI-1640, a serial dilution of 5-flurouracil (50 mg/ml) was produced. This solution may be kept stable at temperatures between 2 and 8°C.

Experimental groups: The untreated SW480 cells in the first group were exposed to RPMI1640 as a control, and the SW480 cells in the second group were exposed to the 5-FU (IC-50). In the third group, SW480 cells were treated with six successive dilutions (500,250,125,62.5,31.2,15.6 µm) of the silibinin stock solution. The treated SW480 cells were incubated for 48 hours after three duplicates of each concentration were administered as monotherapy. The fourth group treated SW480 cells by combining six successive dilutions of silibinin (500,250,125,62.5,31.2,15.6 µm) with the IC50 of 5-fluorouracil from the stock solution. As combination treatment, three duplicates of each concentration were used, and the treated cells were then incubated for 48 hours.

Viability test

The MTT test was used to determine the cytotoxicity of silibinin and the IC50 of 5-FU. The SW480 cells were treated with silibinin at successive dilutions as a single agent and in combination with IC-50 OF5-FU for 48 hours.

After this, the RBMI was removed, and each well was washed with PBS (phosphate buffer saline). After incubation 200µl of MTT solution (0.5mg/ml) at 37°C for three hours, the purple colour of formazan crystals was visualised using an inverted microscope. Next, 100µl of DMSO was added to each well to dissolve the formazan crystals. The plate was incubated at room temperature for 30 minutes, allowing the cells to lyse and the purple crystals to disintegrate. The absorbance was then measured using a microplate at "570 nm."-spectrophotometer [15].

apoptotic markers by ELISA assay

To measure apoptotic markers(casp-3 and BCL-2) using an ELISA assay, sw480 cells were seeded in a 96-well plate and incubated at 370°C overnight with culture media. The cells were then exposed to 5-FU(IC50) (300µm) alone, six serial dilutions of silibinin, and untreated sw480 cells as controls. Each concentration had three replicates, and the cells were incubated at 370°C for 48 hours. The next day, the cells were visualised using an inverted microscope. employs the Sandwich-ELISA type, in which each plate is pre-coated with biomarker-specific antibody, The method was carried out in accordance with the company's instructions after supernatants were applied to the micro ELISA plate wells that bound to certain antibodies.

Intracellular *rhodamine123 accumulation by fluorescence micro plate quick reader assay:*

According to the company's instructions, a stock solution of 123Rhodamin was made by dissolving 2 mg in 1 ml of DMSO and stirring with a Vortex mixer for 5 minutes. This produced a clear solution that was ready for use right away. After the fluorescence microplate fast reader was optimised to determine the ideal rhodamin 123concentration, 5 µm was chosen to be employed for the experiment's subsequent phase. The human colorectal cell line (sw480) was then seeded and silibinin at two different concentrations (500 and 15 µm), 300 µm 5-FU (IC50) as monotherapy, combination therapy of each concentration of silibinin and 300 µm 5-FU (IC50), and the untreated control sw480. Three duplicates of each concentration were used and incubated for 48 hours at 37oC. The old medium was discarded the next day, and 5 µm of 123rhodamine working solution was added to each well. The wells were incubated for 30 minutes before being rinsed twice with PBS. Data was obtained directly from the microplate reader after a few minutes of adjusting it to wave length excitation and emission wavelengths (508-530 nm).

pi3k/Akt/m TOR gene expression by qPCR:

Culture flasks with a density of 0.7*106, seeded with SW480 cells, and exposed to 15, 500µm silibinin as monotherapy, combination therapy of each two doses with 5-FU(IC50), just 5-FU(IC50), and untreated SW480 control group. Each group received four replications, which were incubated at 37°C for 24 hours. The following day, the flask's contents were transferred to conical tubes, centrifuged (1000 rpm for 5 minutes), trypsin was applied to harvest the cells, and PBS rinsed. After extracting the media and preserving the pullet, the contents are transferred to an Eppendrof tube for centrifugation to remove the buffer, and the tubes are then stored at liquid nitrogen (-800c) to prepare them for qpcr. The Easy-spinTM (DNA free) total RNA extraction Kit is used for total RNA extraction, followed by cDNA synthesis in accordance with the kit's instruction protocol. The procedures were carried out in accordance with the

company's instructions using the Add Script cDNA Synthesis Kit.

primers used in this study **(AKT1)** F:TTCTGCAGCTATGCGCAATGTG R:TGGCCAGCATACCATAGTGAGGTT. **(mTOR-C1)** F:GCTTGATTTGGTTCCCAGGACAGTR:GTG CTGAGTTTGCTGTACCCATGT.) **(PIK3CA)**F:GGTTGTCTGTCAATCGGTGACT GT. R:GAACTGCAGTGCACCTTTCAAGC. **(GAPDH)** F:GGAGTCAACGGATTTGGT. R:GTGATGGGATTTCCATTGAT. The steps of Protocol of" GoTaq® RT-qPCR System for Real-Time qPCR" was done according to the manufactures.

Ct values of the genes (calculated automatically by the Mx3005P Stratagene system), "Fold change of the genes (calculated by 2^-ΔΔCT method)"

Statistical analysis of data: the results were express as mean ±SD by graph pad prism 9. To evaluate significant differences between the data means, one-way analysis of variance (ANOVA) for multiple comparisons was utilized, P-values of less than 0.001 and 0.05 were deemed statistically significant

Results

1. Impact of Silibinin on Viability of SW480 Cell Line as Monotherapy and Combination Therapy with 5-FU (IC-50).

There is highly significant ($p < 0.0001$) decrease in the cells growth exposed to all concentrations of Silibinin as monotherapy and combination therapy with 5-FU (IC50) in comparison with control group. As in figure (1) showed the effect of silibinin as monotherapy and combination with 5-FU 300 µm (IC-50) on SW480 cell viability

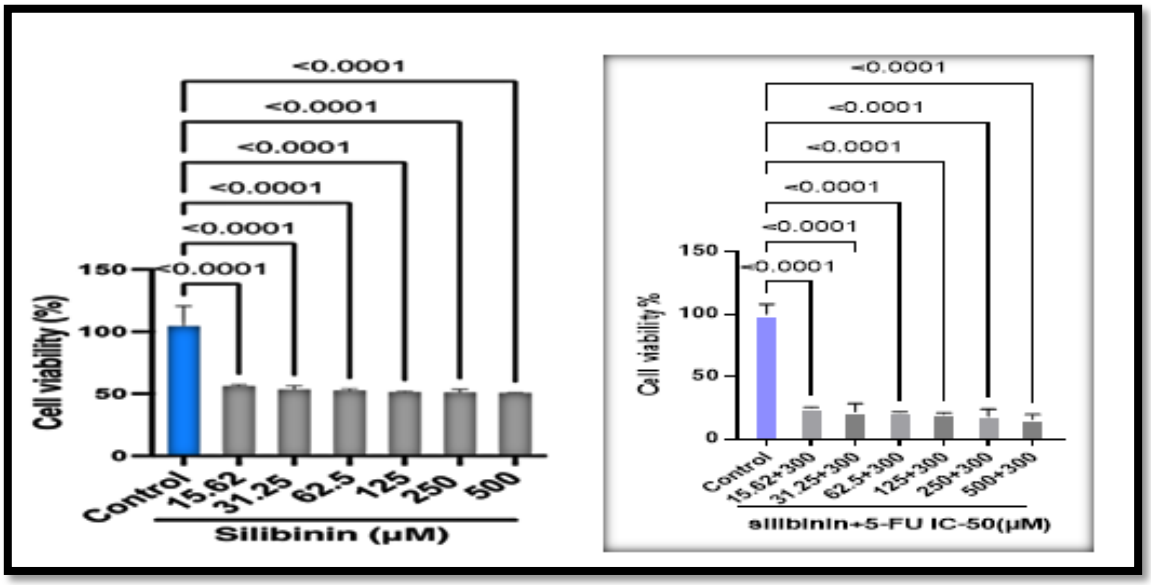

Figure (1) State the impact of Silibinin on SW480 viability monotherapy and combination with 5-FU 24hrs.incubation

2. Antioxidant Capacity of Silibinin in SW480 as Monotherapy and Combination Therapy with 5-FU (IC-50) there was significantly high ($p<0.0001$) increase of antioxidant for all concentrations of silibinin in comparison to both control and 5- FU (IC50) groups. Whereas there was no significant($p>0.05$) difference between 5-FU (IC50) and control group. As in figure (2)

showed the effect of silibinin combined with 5-FU 300 µm (IC-50) on total antioxidant capacity in SW480.

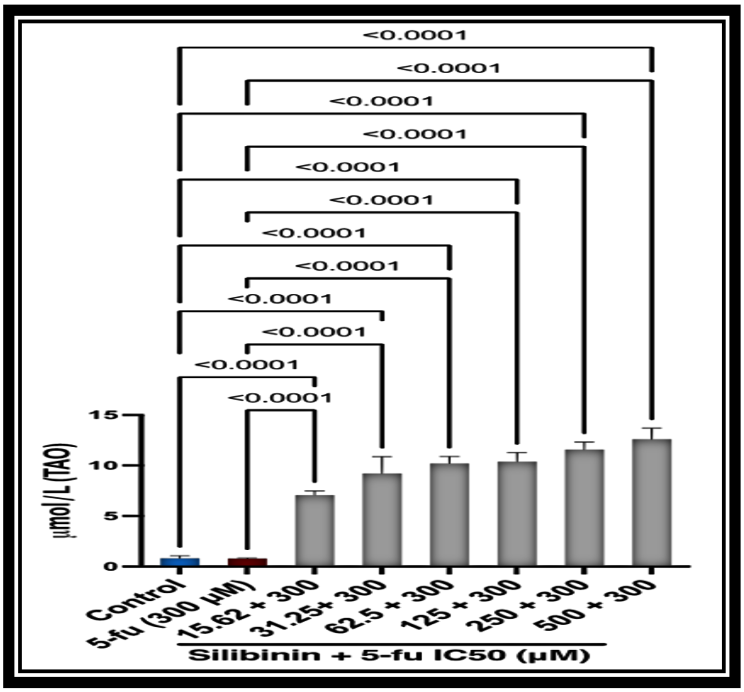

Figure (2)State the antioxidant capacity of Silibinin in SW480 as combination therapy with 5-FU(IC-50) after 48 hours-of incubation

3. Impact of Silibinin on Caspase-3 and BAX protein in SW480

There is highly significant ($p<0.0001$)increase in caspse-3 in the combination therapy of Silibinin with 5-fu(IC-50) for all concentrations of Silibinin in comparison with both control and 5-FUgroups. While there was no significant ($p>0.05$) difference between 5-FU(IC-50) and control group. The figure (3) state the effect of silibinin combined 5-FU 300 µm (IC-50) on level of both proapoptotic Csapaes-3 and antiapoptotic Bcl-2 in SW480.

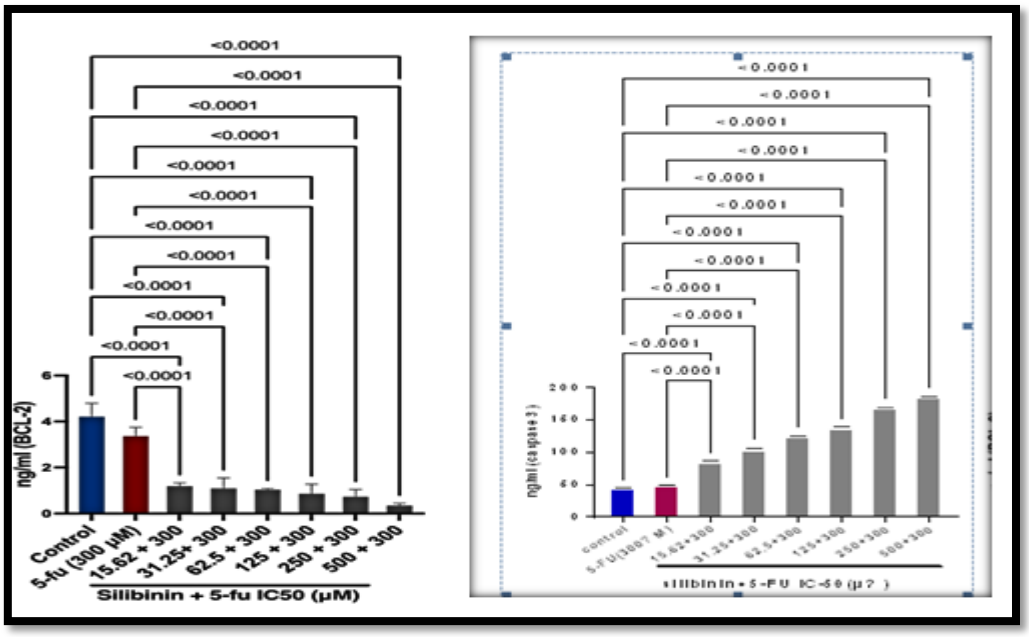

Figure (3) State the impacts of Silibinin on caps-3 and Bcl-2 in SW480

4. Effect of Silibinin on Intracellular Accumulation ofRhodamin123 Florescence dye in SW480 as Monotherapy and Combination Therapy with 5-Fu (IC-50)

There was significant increase of rhodamine 123 accumulation in SW480 cell line exposed to combination therapy of each concentration of Silibinin with 5- FU(IC-50) in comparison with that for both control and 5-FU(IC-50) groups. Whereas there was no significant ($p>0.05$) difference in mean of rhodamine123 accumulation in SW480 cell line between 5-FU(IC-50) and control group. As in figure (4) showed the impact of silibinin combined with 5-FU 300 μm (IC-50) on intracellular accumulation of rhodamine 123 in SW480.

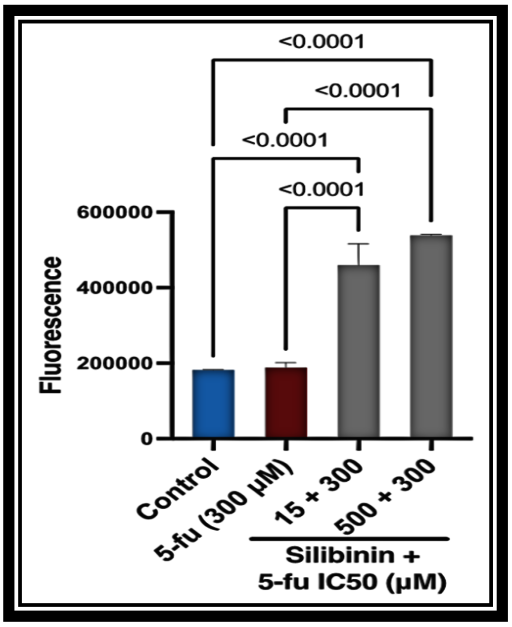

Figure (4): State the impact of Silibinin on intracellular accumulation of rhodamin123 in SW480 cell line as combination therapy with 5-FU (IC-50) after 48 hours-of incubation

5. Impact of Silibinin on PI3KCA,AKT and mTOR Genes Expression in SW480 as Monotherapy and Combination Therapy with 5-fu(IC-50)

When we compared to the control group, there was no significant($p>0.05$) difference in folding changes of PI3KCA gene in SW480 exposed to Silibinin (15 or 500 µM) ,and significantly($p<0.05$) decrease the folding of PI3KCA for combination therapy with 5-FU. While 5-FU(IC50) showed significant ($p>0.05$) enhance folding change of PI3KCAgene. But when compared all groups of Silibinin with 5-FU, it showed that there were highly significant reduction ($p<0.001$)in folding change in PI3KCA gene. There was significant reduction ($p<0.05$) in folding change of AKT gene in SW480 cell line in comparison to both control and 5-fu

(IC50) groups. all groups of Silibinin significant decrease ($p>0.05$) in folding change of mTOR gene in SW480cell line when compare to both control or 5-FU(IC50) groups. As in figure (5) showed the impact of silibinin as monotherapy and combined with 5-FU 300 µm (IC-50) on reduction of relative folding of PI3KCA,AKT and m TOR genes in SW480 cell line.

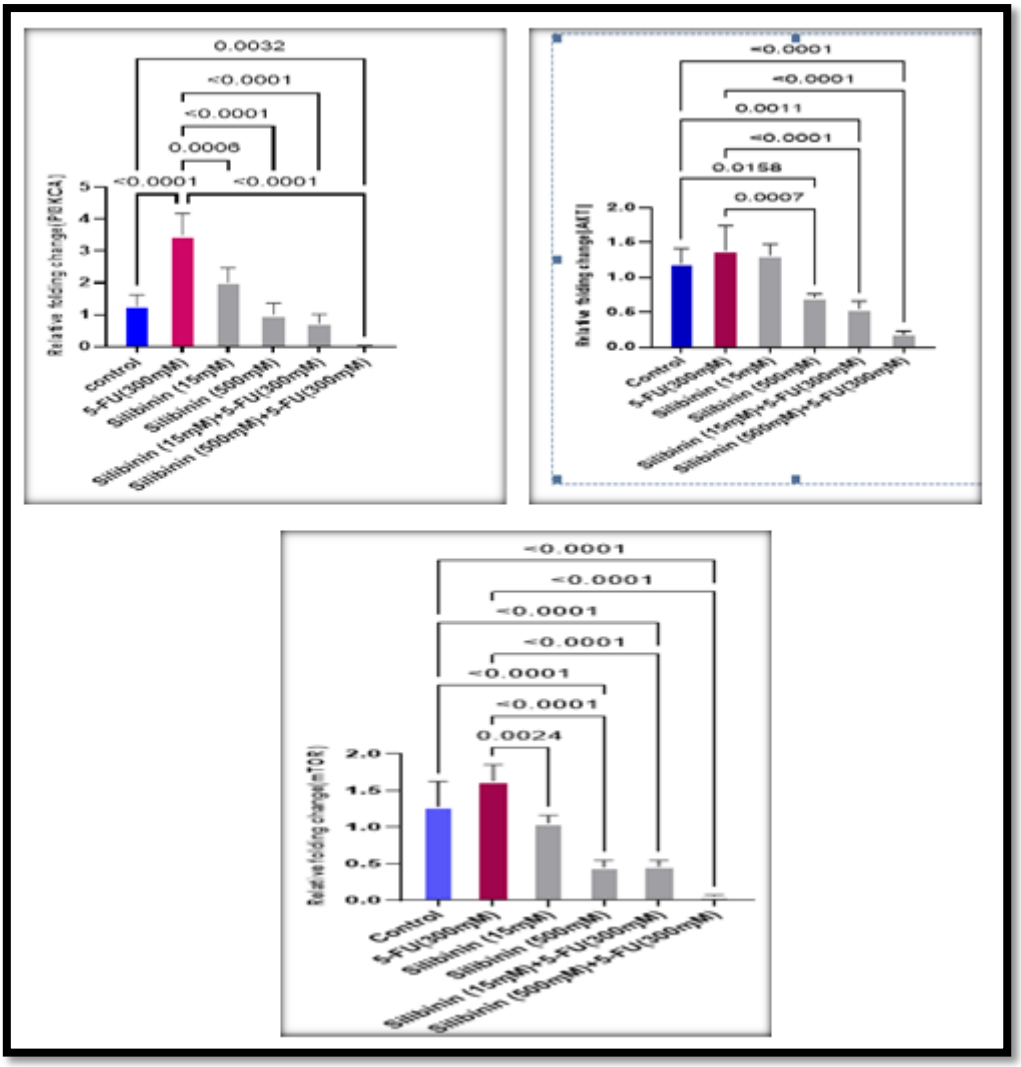

Figure (5): State the impact of Silibinin on PI3KCA,AKT and mTOR Genes Expression in SW480 as Monotherapy and Combination Therapy with 5-FU 300 μm (IC-50)

Discussion

Silibinin could cause reduction in cell viability that was obvious for all concentrations of Silibinin and the best effect was at 500µM. Moreover, the inhibitory effect of 5- FU(IC-50) might enhance when it combined with silibinin which was clear for all concentrations of silibinin and the inhibitory effect of silibinin was doses dependent. These results reflected that investigated the molecular target of Silymarin using in vivo and in vitro model in oral cancer which showed that Silymarin could targeting DR5/caspase-8 apoptotic signaling causing reduction in cell proliferation, enhancing apoptosis and decline the growth of tumor [19]. the combination therapy of Silibinin with 5-FU(IC50) could boost caspase-3 more than monotherapy that suggested the beneficial effect of combination therapy to suppress the proliferation and induction apoptosis of cancer cells. This finding was support evidence from previous observations by a study suggested that Silibinin might trigger cell death by initiating mitochondrial apoptosis, induce autophagy in several kinds of cancer cells, and boosted the mRNA levels of pro-apoptotic proteins Bax and Caspase-3 [20].

The current study related to the effect of silibinin Bcl-2 seem to be consistent with other research

who studied role of Silibinin in oral cancer showed that apoptosis-related proteins accumulated after Silibinin treatment, increasing the proportion of apoptotic cells in a dose-dependent manner. Silibinin significantly enhanced the expression of P53 tumor suppression gene, activation of caspase-3 , up-regulation of Bax and down regulation of Bcl-2 (21).

SW480 cells exposed to two different concentrations of Silibinin (15 and 500µM), it was found that Silibinin might cause a notable retention of rhodamine 123 florescence dye, which explained the role of flavonoids to act as a modulator of p-gp; one of key moderators that responsible for efflux mechanism of drugs outside the cells lead to resistance of cancer cell to anticancer agents which demonstrated in this study through the expulsion of 5-FU when these cancer cells exposed to 5-FU as single therapy this reflected the resistance mechanism of these cancer cells. The benefit behind the combination therapy clearly observed when these cancer cells was exposing to combination therapy of Silibinin both high and low doses with 5-FU in which the retention of rhodamine dye was obviously increased.

this finding supported a study on breast cancer cell line was the model of their study, their finding revealed the role of Silymarin to counteract multidrug resistance caused by P-gp, by using different types of breast cancer cell line which explain that the response of cancer cells to combination therapy of anticancer drug and flavonoids might be differ according to type of cell cultures that either exhibit highly expressed P-gp or not [22].

In this research it was estimated the effect of the exposing SW480 cancer cells to two different ranges of concentrations of Silibinin as monotherapy and combination with 5-FU(IC-50) on PI3kCA gene expression, the result found that combination therapy of Silibinin for both high and low doses with 5-FU could reduce folding changes of PI3KCA gene when compared with folding of 5-FU(IC50). Whereas only the high concentration of Silibinin (500 µM) combined with 5- FU showed notable reduction in folding changes as compared with untreated control cancer cells.

These results agreed with study in which the researcher used bladder cell as model for cancer cells the evaluation of PI3KCA protein was by western blot analysis, their findings revealed that Silibinin even in low doses (10 µM) suppressed the actin cytoskeleton and PI3K/Akt signaling pathway in that cancer cells[23].

Another study that agreed with present findings which clarified the synergistic effect of Silibinin and Sorafenib or Gefitinib on hepatocellular cancer. HCC cells treated with a combination of Silibinin and Sorafenib showed enhanced inhibitory effects by suppression of EGFR-dependent Akt signaling which might be the possible cause of growth suppression of HCC[24].

The justification for these diverse responses was due to intricate relationships between plant chemicals and cancer cells biology, numerous bioactive compounds found in plant extracts could interact with certain cellular pathways to alter gene expression in different ways.

References

[1] Karim ,S.; S. Burzangi ; Ahmad ,A.; Siddiqui ,N.; Ibrahim M.I.; Priyanka, S.; . Abualsunun,W.; and Gabr,G.; PI3K-AKT Pathway Modulation by Thymoquinone Limits Tumor Growth and Glycolytic Metabolism in Colorectal Cancer

.Int. J. Mol. Sci. 23, 2305, 2022

[2] Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, CA. Cancer J. Clin., 66, 7–30. 2016

[3] Strum, W.B. Colorectal adenomas. N. Engl. J. Med. 374, 1065–1075, 2016.

[4] D. B. Longley and P. G. Johnston, "Molecular mechanisms of
drug resistance," Te Journal of Pathology, vol. 205, no. 2, pp. 275–292, 2020.

[5] Wei, J.; Gou, Z.; Wen, Y.; Luo, Q.; Huang, Z. Marine compounds targeting the PI3K/Akt signaling pathway in cancer therapy. Biomed. Pharmacother. 129,p: 110484, 2020.

[6]Jiang, N.; Dai, Q.; Su, X.; Fu, J.; Feng, X.; Peng, J. Role of PI3K/AKT pathway in cancer: The framework of malignant behavior. Mol. Biol. Rep. 47, p:4587–4629, 2020.

[7] Nathan, C.; Cunningham-Bussel, A. Beyond oxidative stress: An immunologist's guide to reactive oxygen species. Nat. Rev. Immunol. 13, p:349–361, 2013.

[8] Sheinman, M.; Hermsen, R. Effects of DNA oxidation on the evolution of genomes. bioRxiv, 2017.

[9] Hassan, M.; Watari, H.; AbuAlmaaty, A.; Ohba, Y.; Sakuragi, N. Apoptosis and molecular targeting therapy in cancer. BioMed Res. Int., 2014.

[10]Zaman, S.; Wang, R.; Gandhi, V. Targeting the apoptosis pathway in hematologic malignancies. Leuk. Lymphoma 55, p:1980–1992. 2014.

[11] Villa-Pulgarín, J.A.; Gajate, C.; Botet, J.; Jimenez, A.; Justies, N.; Varela-M, R.E.; Cuesta-Marbán, A.; Müller, I.; Modolell, M.; Revuelta, J.L.; et al. Mitochondria and lipid raft-located FoF1-ATP synthase as major therapeutic targets in the antileishmanial and anticancer activities of ether lipid edelfosine. PLoS Negl. Trop. Dis. 11, e0005805, 2017.

[12] Jakubowska K, Guzinska-Ustymowicz K, Famulski W, Cepowicz D, Jagodzinska D, Pryczynicz A. Reduced expression of caspase-8 and cleaved caspase-3 in pancreatic ductal adenocarcinoma cells. Oncology letters.; 11(3), p:1879–84, 2023.

[13] Singh RP, Dhanalakshmi S and Agarwal R: Phytochemicals as cell cycle modulators-a less toxic approach in halting human cancers. Cell Cycle 1, p: 156-161, 2002.

[14] Fallah, M., Davoodvandi, A., Nikmanzar, S., Aghili, S., Mirazimi, S. M. A., Aschner, M.,
Naghsh, N. Silymarin (milk thistle extract) as a therapeutic agent in
gastrointestinal cancer. Biomedicine & Pharmacotherapy, 142, Article 112024, 2021.

[15] Bahuguna, A., Khan, I., Bajpai, V. and Kang, S. MTT assay to evaluate the cytotoxic potential of a drug. Bangladesh J Pharmacol., 12(2), p:115-118, 2017.

[19] Won, D.-H., Kim, L.-H., Jang, B., Yang, I.-H., Kwon, H.-J., Jin, B., Cho, S.-D. In vitro and in vivo anti-cancer activity of silymarin on oral cancer. Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine; 40(5):776170, 2018.

[20] Sameri, S., Mohammadi, C., Mehrabani, M., & Najafi, R. Targeting the hallmarks of cancer: the effects of silibinin on proliferation, cell death, angiogenesis, and migration in colorectal cancer. BMC Complementary Medicine and Therapies; 21(1) p:160, 2021.

[21] Zhang, H., Kim, H., Kim, S.-Y., Hai, H., Kim, E., Ma, L., Kim, M. O. Silibinin induces oral cancer cell apoptosis and reactive oxygen species generation by activating the JNK/c-Jun

pathway. Journal of Cancer; 14(10):1875–1887, 2023.

[22] Miglė Paškevičiūtė , Vilma Petrikaitė. Effect of natural flavonoids to reverse P-glycoprotein-related multidrug resistance in breast cancer cell cultures: Am J Cancer Res;12(6), p:2526-2538, 2022

[23] Imai-Sumida, M., Chiyomaru, T., Majid, S., Saini, S., Nip, H., Dahiya, R., Yamamura, S. Silibinin suppresses bladder cancer through down-regulation of actin cytoskeleton and PI3K/Akt signaling pathways. Oncotarget;8(54), p:92032–92042, 2017.

[24] Gu, H. R., Park, S. C., Choi, S. J., Lee, J. C., Kim, Y. C., Han, C. JKim, Y. J. (Eds.).). Geum Youb Noh1, So Hyeon No2,3, and Jae-Hoon Jeong2,3. Combined treatment with silibinin and either sorafenib or gefitinib enhances their growth-inhibiting effects in hepatocellular carcinoma cells. Clinical and Molecular Hepatology, 21 p:49–59, 2015.