The cytotoxic effect of silymarin and 5-FU on three types of cancer cell lines

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Background: Silymarin is a compound derived from milk thistle plant with antioxidant and antinflammatory properties and possibility in having anticancer effect.

Aim of study: To examine the cytotoxic effect of silymarin on three types of cancer cell lines in compares to 5-Flurouracil (5-FU).

Materials and methods: a study was performed on the three cancer cell lines Rhabdomyosarcoma (RD) cell line, Glioblastoma multiform primary culture. (AMGM) human cervical cancer cell line (Hela) and Rat Embryo Fibroblast (REF) as normal cell line. cell lines were measured at 24, 48, and 72-hour in a microtitration plate under complete sterile conditions. The Cells were grown in optimum condition at 37c° and 5% CO_2. Different concentrations of both silymarin and 5-FU and their combination was used starting from (25 to 400) µg/ml of two fold dilution for each concentration were prepared and tested on each cell line, with three replicates for each concentration. MTT (3, 5-(Dimethylthioazol-2-yl)-2, 5-diphenyltetrazolium bromide) method used to detect the cytotoxicity of each drug and their combination.

Results: The obtained results showed a potent cytotoxic and growth inhibitory effect of silymarin on cancer cell with no cytotoxic effect on normal cells in comparison to 5FU *in vitro*. The effect of combination

of 5-FU with silymarin on cancer cell lines was showed synergistic effects in concerning to the cytotoxicity of both.

Conclusion: Silymarin showed a promising anticarcinogenic activity as well as increased the cytotoxic activity of 5-FU when combined with it. Silymarin is a safe and can be used as adjuvant in cancer therapy.

Key wards: silymarin, 5-FU, cell line, growth inhibition.

الفعالية المضادة للسرطان لكلا السليمارين والفايف فلورويوراسيل على ثلاثة انواع من خطوط الخلية السرطانية

الأستاذ د.فاروق الجواد ، الأستاذ د.شلال مراد ، الصيدلي رضا رجا معيوف

الخلاصة :-

السليمارين مركب نباتي مشتق يمتلك تأثيرات مضادة للأكسدة والالتهاب واحتمالية امتلاكه فعالية مضادة للسرطان من خلال تطبيقه على ثلاثة خطوط للخلية السرطانية وخط اخر اخر طبيعي استعمل كسيطرة .

لقد درست تأثيرات السليمارين مقارنة مع ٥-فلورويوراسيل استعمل منفردا او متحدا على خطوط الخلية السرطانية الثلاثة عندما خففنا بتراكيز من ٢٥-٤٠٠مايكروغرام وكان التخفيف بنسبة ضعفين لكل تركيز حيث كان التعرض على الخلايا لفترة ٢٤-٤٨ او ٧٢ ساعة قيست بطريقة ال(م.ت.ت) وعلى صفيحة المعايرة الصغرى وتحت ظروف معقمة .

اظهرت النتائج ان للسليمارين تأثيرا مضادا للسرطان ومثبطا للنمو على خطوط خلية السرطان الثلاثة وبدون اي تأثيرعلى خط الخلية الطبيعية مقارنة مع فلورويوراسيل كما وجد ان هنالك تأثيرا تأزريا وزيادة في الفعالية المضادة للسرطان عندما استعملا سوية بالزجاج.

بينت النتائج امكانية االستعمال السليمارين كعقار مساعد امين لمعالجة السرطان عندما استعمل سوية مع الفلورويوراسيل.

الكلمة المفتاح :السليمارين ، ٥-فلورويوراسيل ، خطوط الخلية ، تثبيط النمو

Introduction:

Cancer is a serious disease predicted to be the second cause of morbidity and mortality and have been increasing in the next few decades in the world due to high incidence of disease annually [(1)]. Anticarcinogenic agents in current use, which includes drugs of microbial origin, and drugs derived from plants. Silymarin is a compound extracted from milk thistle plant where sililbinin is a flavonolignan more active of silymarin [(2)] , the later used as antioxidant agent with hepatoprotective activity[(3,4)] A groups of researchers [(5)] reported that sililbinin main component of silymarin exerts antiangiogenic effect through decrease the expression of angiogenic stimulants HIF-1α (necrosis factor) INOS, PECAM-1, VEGF (Vascular endothelial growth factor receptor). The present study was performed to examine the cytotoxic activity of silymarin on cancer cell lines with or without 5-FU agent. Indeed there are no differences in the activity of silymarin and silybin against tumor cells when used experimentally *in vivo* and *in vitro* [(6)].

Materials and methods

Silymarin was used a pure powder and 5-FU vial in the present study. The three cancer cell lines Rhabdomyosarcoma (RD) cell line, Glioblastoma multiform primary culture. (AMGM) human cervical cancer cell line (Hela) and Rat Embryo Fibroblast (REF) as normal cell line. (RD,AMGM and Hela) and normal REF cell line obtained from Iraqi Center for Cancer and Medical Genetics Research (ICCMGR) in addition to RPMI media and other solutions used in the study. The periods of exposure of silymarin and 5-FU on cell lines were measured at 24-hour, 48-hour, and 72-hour in a microtitration plate under complete sterile conditions. The Cells were grown in optimum condition at 37c° and 5% CO_2. Different concentrations of both silymarin and 5-FU and their combination was used starting from (25 to 400) µg/ml of two fold dilution for each concentration were prepared and tested on each cell line, with three replicates for

each concentration. MTT (3, 5-(Dimethylthioazol-2-yl)-2, 5-diphenyltetrazolium bromide) method used to detect the cytotoxicity of the compound *in vitro* study.

Results

The effect of silymarin appears very clearly on AMGM cancer cell line. The minimum inhibitory effect detected of silymarin is (7.07%) at 25µg/ml in 24hr incubation time in compare to (42.04%) in 24hr of incubation of 5-FU at 25 µg/ml concentration used table(1).

Table-1- Cytotoxicity of silymarin and 5-FU on AMGM cell line*

AMGM cancer cell line	Conc. µg/ml	Silymarin			5-FU		
		24hr	48hr	72hr	24hr	48hr	72hr
	Conc.400	24.07	39.79	52.92	62.54	71.41	83.93
	Conc.200	17.76	30.17	50.71	51.35	66.25	81.04
	Conc.100	13.99	24.26	40.36	49.82	64.88	74.08
	Conc.50	9.21	23.92	38.12	42.34	49.62	71.69
	Conc.25	7.07	10.65	23.64	42.04	37.18	58.48

*All results are significant when $P<0.05$

The inhibitory growth effect of silymarin on AMGM reach to (52.92%) in 72hr of incubation at 400 µg/ml in comparison to 5-FU which reach to (83.93%) in 72hr of incubation at 400 µg/ml concentration. The increase in the efficacy of 5-FU when combined with same dose of silymarin clearly detected as synergism effect of silymarin more appear at 100µg/ml than 50µg/ml concentration used. The cytotoxicity% of 5-FU increased from 24.36% at concentration 100µg /ml of 5-FU to 34.93% when combined with silymarin at concentration 100µg /ml in 24hr period of incubation. Also at the

concentration 50µg/ml of 5-FU, the cytotoxicity raise from 45.44% to 60.77% when combined with 100µg/ml concentration of silymarin in 72hr period of incubation table (4).

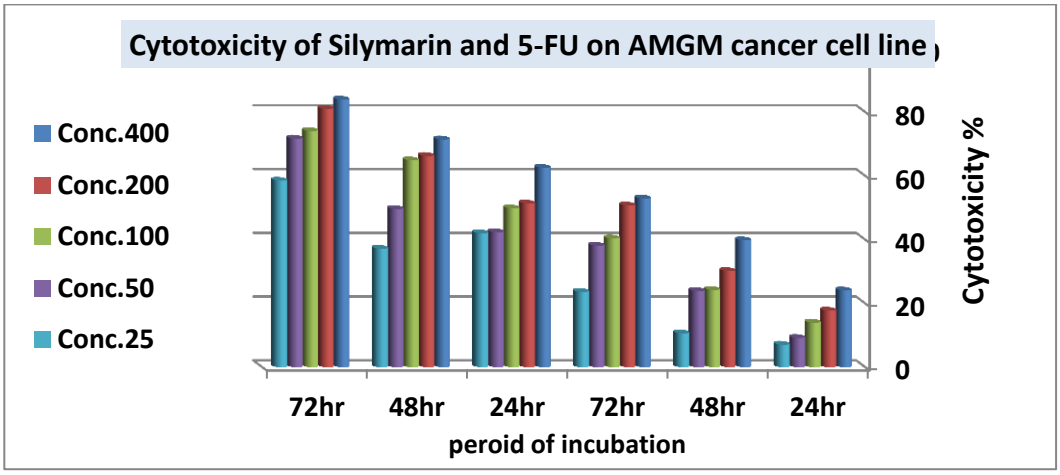

Figure (1) Cytotoxicity of silymarin and 5-FU on AMGM cell line. conc.: concentration µg/ml

The inhibitory effect of silymarin on Hela cancer cell was started from 3.01% at 25µg/ml
in 24hrs of incubation until reach 57.44% at 400ug/ml in 72hrs of incubation in comparison to 5FU was started from 33.72% at 25µg/ml in 24hrs of incubation and reach to 82.92% at 400µg/ml in 72hrs table (2).

Table 1-2 Cytotoxicity of silymarin and 5-FU on Hela cell line*

Hela cancer cell line	Concentration µg/ml	Silymarin			5-FU		
		24hr	48hr	72hr	24hr	48hr	72hr
	Conc.400	23.77	33.43	57.44	47.70	66.85	82.92
	Conc.200	10.71	21.97	51.79	47.21	60.00	81.59
	Conc.100	20.22	19.42	51.95	43.55	54.93	77.85
	Conc.50	13.88	17.18	53.79	35.36	21.88	75.79
	Conc.25	3.01	15.68	47.79	33.72	17.18	73.85

*All results are significant when $P<0.05$

While when used silymarin in combined dose with 5-FU was lead to increase the cytotoxicity from 67.34% at 100µg/ml 5-FU alone to 68.98% at combination (100µg5-FU/ml with 100µg silymarin/ml) in 72hr period of incubation tab

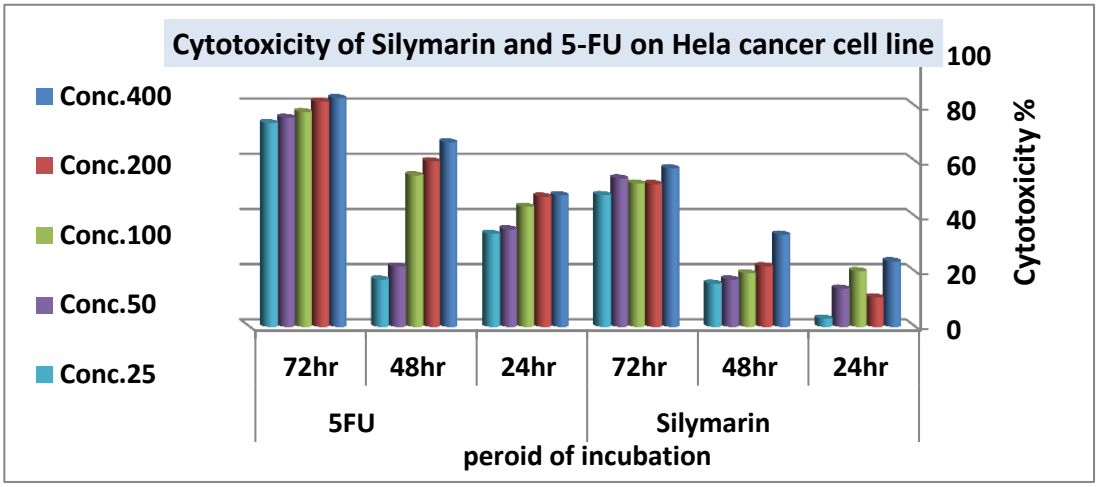

Figure -2- Cytotoxicity of silymarin and 5-FU on HeLa cell line. Conc.(concentration µg/ml)

Table -3- Cytotoxicity of silymarin and 5-FU on RD cell line*

Table 1-3 Cytotoxicity of silymarin and 5FU on RD cell line

RD cancer cell line	Concentration µg/ml	Silymarin			5-FU		
		24hr	48hr	72hr	24hr	48hr	72hr
	Conc.400	33.82	39.79	52.92	53.77	71.41	83.93
	Conc.200	30.80	30.17	50.71	48.09	66.25	81.04
	Conc.100	26.95	24.26	40.36	41.13	64.88	74.08
	Conc.50	24.11	23.92	38.12	36.76	49.62	71.69
	Conc.25	17.88	10.65	23.64	24.71	37.18	58.48

*All results are significant when $P<0.05$

The cytotoxic effect of silymarin on RD cell line started from (17.88%) at 25 µg/ml in 24hr incubation time, and increased to reach (52.92 %) at 400µg/ml in 72 hrs time. Where is combined silymarin with 5-FU, the efficacy increase to reach (74.57%) in
compare to (67.34%) of 5-FU alone at the same time of incubation 72hr.

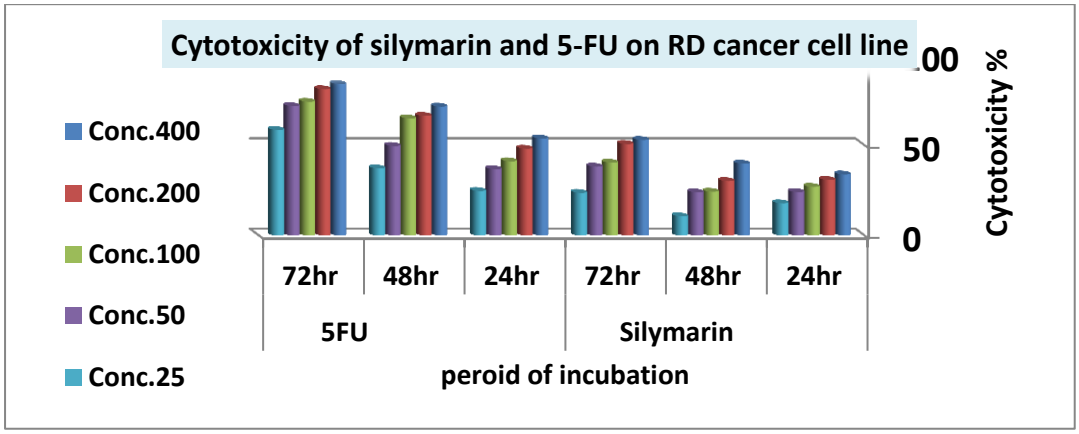

Figure -3- Cytotoxicity of silymarin and 5-FU on RD cell line

icity of combination of silymarin and 5-FU on three types of cancer cell lines

Concentration µg/ml	Cytotoxicity on AMGM			Cytotoxicity on Hela			Cytotoxicity on RD		
5-FU/sil (µg/ml)	At 24hr	At 48hr	At 72hr	At 24hr	At 48hr	At 72hr	At 24hr	At 48hr	At 72hr
5-FU/sil 100/100	36.93*	53.79*	61.89*	15.30*	59.34*	68.98*	30.53**	53.15*	74.56**
5-FU/sil 100/50	28.55**	49.33**	59.16*	14.64**	57.32*	69.36**	21.93**	59.82*	69.36**
5-FU/sil 50/50	25.75*	43.02*	57.42*	10.27*	55.05*	65.70*	21.42**	48.22*	53.17**
5-FU/sil 50/100	23.96*	46.67**	60.77**	8.47*	49.24**	61.27*	21.93**	47.31*	61.27**
5-FU 100	24.30**	40.78**	50.35**	9.84**	57.20**	67.34**	15.96**	51.23**	67.34**
5-FU 50	17.04**	32.55**	45.44**	5.46*	49.62**	61.56**	8.25**	47.40**	61.56**
Sil 100	17.11**	26.74**	28.55**	3.28*	18.94**	38.15**	5.96**	26.21**	38.15**
Sil 50	15.70**	17.91*	13.41*	2.19*	9.72**	21.77*	3.16*	12.77**	23.41**

5-FU:5-flurouracil , sil: silymarin , hr: hour.

*Results are significant when $P<0.05$

**Results are highly significant when $P< 0.001$

As show in the figure the cytotoxic effect of both 5-FU and silymarin increase as the time of incubation increased.

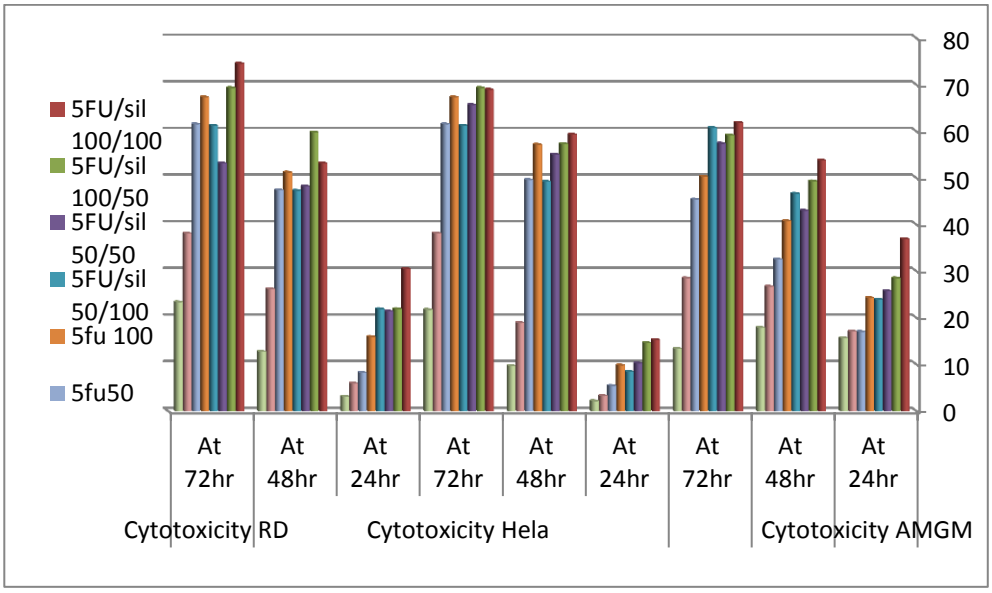

Figure-4-cytotoxicity of combination of silymarin and 5-FU on three types of cancer cell lines.

5-FU: 5-flurouracil, sil: silymarin, hr:hour, AMGM, RD, Hela : cancer cell lines

Also the results was showed the viability of normal cell rat embryo fibroblast (REF) didn't decrease when incubated with silymarin at different time (24hr, 48hr, 72hr).

Discussion

The severe toxicity of most anticarcinogenic drugs accelerate to search for new drug and efforts for development newer agents, that can prevent or slow-down cancer growth with less toxicity and more safety. Silymarin was one of these agents. Its contains silybin A and B, silychristin, and less silydanin which is extract from the Silybum marainum plant. These Phytochemicals derived from the milk thistle that has more attention in last decades. These agents have antioxidant, antinflammatory and cytoprotective properties that been more beneficial in human [(4) (8)].

The beneficial anticarcinogenic of silymarin was shown in elevation the bioavailability of cytotoxic drugs such as daunomycin, vinblastine, and doxorubicin in cancer cells by inhibition of P-glycoprotein (P-gp), MRP1 -mediated drug carrier and breast cancer resistance protein (BRCP)[(9)(10)]. Our results are similar to the results of these where silymarin used with 5-FU.

5-Fluorouracil is a pyrimidin analaong reached to high cytotoxic efficacy about 83% at 400 g/ml in 72 hour duration of incubation also in turned on cancer cell lines through conversion to 5-FdUMP which competes with deoxuridine monophosphate (d-UMP) for eoxy thymidin synethatase thus depriving the cell of thymidin ,one of the essential precursor for DNA synthesis[(11)].

Silymarin was demonstrated to have wide range of cytotoxic activity against the three types of cancer cell lines (AMGM, RD, Hela) but RD cancer cell line was the more sensitive to the cytotoxic effect of silymarin. The cytotoxicity of the drug increase as increase the concentration and time of incubation increased. This effect is related to antiproliferative activity and induced apoptotic death of cancer cell [(12) (13)] which is similar to our results.

The variation in the percentage of cytotoxic efficacy of silymarin that had been obtained in the present study at three times of duration of incubation at five different concentrations suggested that silymarin

effect may be involved in more than one mechanism on the cancer cells due to different concentrations of silymarin and have different intensive action on cancer cells as efficacy depend on concentration and time of exposure.

The obtained results revealed that silymarin potentiate the cytotoxic activity of 5-FU on the cancer cell lines (AMGM, RD and Hela) when used in different concentration ratio. Silymarin interfere with the integrity of cell membrane function so potentiate the action of 5-FU through decrease it exclusion out the cell. Our results similar to the results of other [14] who shown that silybin strongly synergizes activity of doxorubicin, cisplatin and carboplatin against human prostate carcinoma.

The absent cytotoxic activity of silymarin on Rat embryo fibroblast (the normal cell line) that used in the present study is compatible with other studies [15] in using silymarin as safe drug for liver protective and treatment of alcohol induced liver damage.

In conclusion : the wide rang activity of silymarin can inhibit the growth of cancer cell lines with percentage of 50-60% in compare to non treated cancer cell may give the chance to the drug to be used as adjuvant in cancer therapy.

References:

1. International agency for research on cancer 2011.
2. Ramasamy K, Agarwal R. Multitargeted Therapy Of Cancer By Silymarin. Cancer Lett 2008; 352-362
3. Comelli, M.C., Mengs, U., Schneider, C., and Prosdocimi, M. (2007). Toward the definition of the mechanism of action of silymarin: Activities related to cellular protection from toxic damage induced by chemotherapy. *Integr. Cancer Ther.* 6, 120-129.
4. Al-Jawad FH, Al-sharlqie K, Nashtar S-B , silymarin as antidote in thallium poisoning in rabbit. J. of AL-Yarmouk 2015.
5. Raina, K., Blouin, M.J., Singh, R.P., Majeed, N., Deep, G., Varghese, L., Glodé, L.M., Greenberg, N.M., Hwang, D., Cohen, P., Pollak, M.N., and Agarwal, R. (2007). Dietary feeding of silibinin inhibits prostate tumor growth and progression in transgenic adenocarcinoma of the mouse prostate model. *Cancer Res*. 67, 11,083-11,091.
6. Rajamanickam, S., Andagarwal, R. (2008). Natural Products And Colon Cancer: Current Status And Future Prospects. Drug Dev. Res. 69, 460–471.
7. Mackinnon Sl, Hodder M, Craft C, Simmons-Boyce J.(2007) Silyamandin, A New Flavonolignan Isolated From Milk Thistle Tinctures.
8. Raina, K., Rajamanickam, S., Singh, R.P., Deep, G., Chittezhath, M., and Agarwal, R. (2008). Stage-specific inhibitory effects and associated mechanisms of silibinin on tumor progression and metastasis in transgenic adenocarcinoma of the mouse prostate model. *Cancer Res*. 68, 6822-6830.
9. Gazak R, Svobodova A, Psotova J, Sedmera P, Prikrylova V, Walterova D, Kren V(2007). Oxidised derivatives of silybin and

their antiradical and antioxidant activity. Bioorg. Med. Chem.;12:5677–5687
10. Saller, R., Brignoli, R., Melzer, J., and Meier, R. (2008). An updated systematic review with meta-analysis for the clinical evidence of silymarin. *Forsch. Komplementärmed*. 15, 9-20.
11. Clark A.M, Finkel.A, Roy A.J and Whalen K.,(2012) anticancer drugs, pharmacology fifth editation P. 491 lippincott, Williams Wilkins.
12. Flaig TW, Su LJ, Harrison G, Agarwal R, Glodé LM. Silibinin synergizes with mitoxantrone to inhibit cell growth and induce apoptosis in human prostate cancer cells. Int. J. Cancer 2007;120:2028–20
13. Kaur, M., Velmurugan, B., Tyagi, A., Deep, G., Katiyar, S., Agarwal, C., Et Al. (2009b). Silibinin Suppresses Growth And Induces Apoptotic Death Of Human Colorectal Carcinoma Lovo Cells In Culture And Tumor Xenograft. Mol. Cancer Ther. 8, 2366–2374.
14. Tyagi A, Sharma Y, Agarwal C, Agarwal R. Silibinin Impairs Constitutively Active Tgfalpha-Egfr Autocrine Loop In Advanced Human Prostate Carcinoma Cells. Pharm Res. 2008 Sep;25(9):2143-50. Epub 2008 Feb 6.
15. Ferenci P, et al 1989 Ferenci P, Et Al. Randomized, Controlled Trial of Silymarin Treatment in Patients with Cirrhosis of the Liver. Hepatol 1989;9:105-13.