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Comparison of the immunosuppressive effects of Silymarin with Rapamycin and FK506 on the proliferation and apoptosis of T cells *in vitro*

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Abstract:

Introduction: Silymarin, as a polyphenolic flavonoid derived from milk thistle (Silybum marianum), is known to have antioxidant, immunomodulatory, antiproliferative, antifibrotic, and antiviral effects. The goal of this study was to determine immunosuppressive effect of Silymarin on proliferation and apoptosis of human T cells in comparison with Rapamycin and FK506.

Methods: Peripheral Blood Mononuclear Cells (PBMCs) from the healthy individuals were activated with Con A (5µg/ml) and then treated with Silymarin, Rapamycin and FK506 at various concentrations (0.001, 0.01, 0.1, 1, 10,100 and 200 µM) for 5 days. PBMCs were examined for proliferation using the CFSE assay and the concentration that inhibited 50% of the cell proliferation (IC_{50}) was determined for each treatment. For apoptosis assay using flow cytometry, PBMCs were activated with Con A and treated with IC_{50} dose of Silymarin, Rapamycin and FK506 for five days, then cell apoptosis was analyzed by FITC-annexin V/PI staining and flow cytometry. The effects of Silymarin, Rapamycin and FK506 on the activation of PARP (poly ADP ribose polymerase) pathway in PBMCs stimulated with Con A and treated with IC50 dose of drugs for five days evaluated using the PathScan cleaved PARP sandwich ELISA kit.

Results: This study showed that Silymarin had the ability to inhibit T cell proliferation *in vitro*. Moreover, our results indicated that 100 µM and 200 µM of Silymarin showed inhibitory effect on T cells proliferation compared with FK506 and Rapamycin. Our data showed that the effective doses (IC_{50}) of Silymarin, FK506 and Rapamycin were 3×10^{-5} µM, 10^{-8} µM and 10^{-6} µM respectively. Data showed that the inhibitory effect of Silymarin, FK506 and Rapamycin on the T cell proliferation was not due to cytotoxicity and none of these drugs at the IC_{50} concentration had no effect on the level of cleaved PARP.

Conclusion: This study shows that Silymarin has the ability to inhibit T cell proliferation *in vitro* and exert immunosuppressive effects. Silymarin could be a good candidate for immunosuppressive therapy for certain medical conditions with superior efficacy and lesser toxicity compared with other immunosuppressive drugs.

Keywords: Silymarin, Immunosuppressive effect, Rapamycin, FK506, CFSE, FITC-annexin V/PI

List of Article:

1- تاثیر داروی سیلیمارین بر تکثیر لنفوسیتهای T فعال شده انسانی درشرایط آزمایشگاهی (*in vitro*)

2- The immunosuppressive effects of Silymarin with Rapamycin and FK506 on the proliferation and apoptosis of lymphocytes

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ABBREVIATIONS:

APC: Antigen-presenting cell

CD: Cluster of differentiation

CSFE: Carboxyfluorsecin succinimidyl ester

ConA: Concanavalin A

DC: Dendritic cell

DMSO: Dimethyl Sulfoxide

ELISA: Enzyme Linked Immuno Sorbent Assay

FKBP12: FK binding protein 12

IC_{50}: Half maximal inhibitory concentration

IFN-γ: Interferon- gamma

Ig: Immunoglobulin

iNOS: Inducible nitric oxide synthase

IL: Interleukin

LPS: Lippolysaccharide

MHC: Major histo compatibility complex

mTOR: Mammalian target of Rapamycin

NF-κB: Nuclear Factor kappa B

PARP: Poly ADP ribose polymerase

PBMC: Pheripheral Blood Mononuclear Cells

PBS: Phosphate Buffered Saline

PHA: Phytohemagglutinin

PI: Propidium iodide

PMSF: Phenyl methyl sulfonyl fluoride

SMAC: Supermolecular activation complex

TCR: T cell Receptor

TNF: Tumor Necrosis factor

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چکیده:

مقدمه: استفاده گسترده از داروهای گیاهی به دلیل اثرات حفاظتی آنها بر ارگانهای مختلف بدن در مطالعات بسیاری نشان داده شده است. سیلیمارین یک کمپلکس فلاونولیگنان مشتق از گیاه خار مریم (milk thistle) با نام علمی Silybum marianum است. از این ترکیب به علت داشتن اثرات ضد التهابی، ضد ویروسی، ضد تکثیری و حفاظتی در بیماریهای کبدی (hepatoprotective) در کلینیک استفاده می شود. اخیرا اثرات تعدیل ایمنی (immunomodulatory) این گیاه از سوی محققین مورد توجه قرار گرفته و در این خصوص مطالعاتی صورت پذیرفته است. هدف از این مطالعه بررسی اثر ایمونوساپرسیو سیلیمارین و FK506 و راپامایسین بر روی تکثیر و آپوپتوز لنفوسیت های T فعال شده انسانی در شرایط *in vitro* بود. **روشها:** سلول های تک هسته ای خون محیطی از خون افراد داوطلب سالم جدا گردید و با کانکاناوالین A (5µg/ml) تحریک شدند سپس با دارو های سیلیمارین، راپامایسین و FK506 در غلظت های (0.001, 0.01, 0.1, 1, 10, 100 and 200 µM) به مدت ۵ روز در شرایط انکوباسیون مجاور شدند. به منظور بررسی اثر تکثیری داروها بر روی سلولهای تک هسته ای خون محیطی از ماده فلورسنت CFSE استفاده شد و هم چنین غلظتی از دارو که باعث مهار ۵۰٪ سلول ها شده بود (IC_{50}) برای هر یک از دارو ها به طور جداگانه محاسبه گردید. برای سنجش میزان آپوپتوز در سلول های T فعال شده با کانکاناوالین A که با دارو های سیلیمارین، راپامایسین و FK506 در غلظت های IC_{50} مجاور شده بودند از رنگ FITC-annexin V/PI استفاده شد و با دستگاه فلوسیتومتری مورد بررسی قرار گرفت. از طرفی میزان بیان مولکول PARP (poly ADP ribose polymerase) در سلول های T فعال شده بعد از ۵ روز انکوباسیون با داروها در غلظت IC_{50} با استفاده از کیت PathScan cleaved PARP sandwich ELISA kit مورد ارزیابی قرار گرفت. **یافته ها:** این مطالعه نشان داد که سیلیمارین توانایی مهار تکثیر سلول های T را در شرایط *in vitro* دارد. نتایج حاکی از آن بود که سیلیمارین در غلظت های 100 µM و 200 µM اثر مهاری قوی تری در مقایسه با راپامایسین و FK506 بر روی تکثیر سلول های T دارد. از طرفی نشان داده شد که دز موثر (IC_{50}) برای سیلیمارین، راپامایسین و FK506 که باعث مهار ۵۰ درصدی تکثیر سلول های T می شود به ترتیب 3×10^{-5} µM، 10^{-6} µM و 10^{-8} µM است. همچنین نشان داده شد که اثر مهاری سیلیمارین، راپامایسین و FK506 به دلیل سیتوتوکسیک بودن این داروها نمی باشد چراکه هیچ یک از داروها در غلظت IC_{50} اثری بر روی بیان مولکول PARP نداشتند. **نتیجه گیری:** باتوجه به اثر مهاری سیلیمارین بر تکثیر سلولهای T ، از این رو شاید بتوان از آن به عنوان یک داروی حیات بخش در شرایطی که نیاز به سرکوب ایمنی است استفاده نمود و جایگزین داروهای شیمیایی ایمونوساپرسیو شود.

کلید واژه ها : سیلیمارین، سلولهای T ، سلولهای تک هسته ای خون محیطی، Carboxyfluorescein- succinimidyl ester (CFSE)، راپامایسین، FK506