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Title:

Investigation of Changes in the Expression of Proinflammatory Cytokines Caused by Extract Silybum marianum L. in In-vitro and In-vivo

Amir Akbarnejad Eshkalak[1], Hossein Maghsoudi[2]
1Department of Biology, Payame Noor University, Tehran, Iran
2Department of Biotechnology, Payame Noor University, Tehran, Iran
Email address: amiir.akbarnejad@gmail.com
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Abstract:

In this paper, the effects of alcoholic extract of *Silybum marianum L. (AESM)* on inflammation and reduction of cartilage destruction in a rabbit model with monosodium iodoacetate (MIA) osteoarthritis were investigated. AESM was able to effectively and dose-dependently suppress the mRNA expression of proinflammatory cytokines, including iL-6, iL-1α, iL-18, and TNF-α in LPS-stimulated synoviocytes. Furthermore, the expression of these genes in blood and plasma was significantly diminished. The effect of AESM was compared with competing for chemical drugs such as dexamethasone and ibuprofen among control and patient groups of rabbits with OA. The middle part of cartilage in rabbits was measured by hematoxylin and eosin (H&E) staining. It was found that AESM has caused the accumulation of indispensable proteoglycans of cartilage. **Background:** Researches indicate that silymarin is a compound that contains various properties like anty-inflammatory hepatoprotective, antioxidant, heart-protective, hypocholesterolemic, anti-diabetic, anticancer, and cardioprotective activities. Clinical studies have been demonstrated that silymarin has very rare side effects at high doses (>1500 mg/day). **Objective:** The main aim of this study was to concentrate on the treatment of OA with the help of drugs with minimal side effects to decrease arthritis following the cessation of proinflammatory enzyme cascades. **Methods:** RNA extraction by RTRIzol method (Carlsbad, Calif., USA), Convert RNA to cDNA (Malaysia - Selangor), evaluation of gene expression by RT-PCR, simulation of OA with the help of MIA, extraction with a rotary evaporator vacuum device, the MTT technique, isolation and culture of RFLS. Cartilage staining by method hematoxylin and eosin (H&E) (Bio-Optica, Italy). In addition (MIA, 4mg/50

μ l, Sigma- Aldrich, MO, USA). **Results:** AESM decreased the expression of iL-6, iL-1 α , iL-18 and TNF- α genes in RFLS cells and in cartilage and were confirmed the results by Real-Time PCR. The AESM almost caused a decrease in the percentage of cells stimulated by 50% which is a significant decrease compared to Dexamethasone and ibuprofen (NSAID). Therefore, it can be a worthy therapeutic purpose for OA patients in the future. Conclusions: AESM can compete meaningfully with drugs such as dexamethasone and ibuprofen in the treatment of OA. Our experiments indicated that consumption administration of AESM reduces the expression of TNF- α , iL-6, iL-1 α and iL-18 genes and can compete well with common drugs (Dexamethasone and Ibuprofen) in the treatment of OA. The effect of AESM intensified with increasing concentration and had no side effects at very high doses.
Keywords: *Silybum marianum L.*, Monosodium Iodoacetate, Pro-inflammatory Cytokines, Osteoarthritis