In-vitro and In-vivo Effects of NEM® on Pro-inflammatory Cytokines

NEM® Brand Eggshell Membrane
Supports Healthy Inflammatory Response

NEM® brand eggshell membrane is a superior joint health ingredient designed to provide rapid resolution of joint discomfort and stiffness associated with sports activities and age-related wear-and-tear. The effectiveness of NEM® supplementation has been demonstrated in three published human clinical trials. Building upon the knowledge gained from these efficacy studies, ESM Technologies began research into the potential mechanisms through which NEM® might exert its positive effects; from this two additional studies have been published.

Human cell-based assays were used in the first published mechanism study, designed to evaluate the immune-modulatory and anti-inflammatory effects of NEM®. It is known that as a normal part of the body’s management of injury, the immune system sends out pro-inflammatory mediators (cytokines) that in turn activate certain peripheral white blood cells (leukocytes) and initiate rapid division of these cells to help with the process of walling off and repairing injured tissue. However, this normal, healthy process can become altered, leading to prolonged inflammation, diminished mobility and discomfort. This altered inflammatory process is the cause of many of the symptoms experienced by those with osteoarthritis.

In this first study, two NEM® preparations were used. One was a simple aqueous solution and the other was NEM® that had been subjected to conditions that simulate the human digestive process. As a control, human peripheral blood was exposed to substances (mitogens) that stimulate cytokine production and leukocyte proliferation. Other blood samples were then either pretreated with the aqueous solution of NEM® or with the NEM® that had been subjected to digestive conditions prior to exposure to the mitogens.

A decrease in the production of three pro-inflammatory cytokines, IL-6, IFN-γ and TNF-α was significant in samples of the aqueous solution of NEM® alone as well as NEM® exposed to conditions mimicking gastrointestinal digestion. Of even greater significance was the discovery that exposure to NEM® reduced these cytokine levels.

to GI digestive conditions enhance the reduction in TNF-α levels. TNF-α is known to attract cell infiltration into joints and contribute to the inflammation within the joint. Therefore this finding is of particular interest in terms of identifying and understanding the mechanisms through which NEM® functions in mediating joint inflammation.

The second study and next step in elucidating the anti-inflammatory action of NEM® was to use an animal model to evaluate the effects of NEM® on pro-inflammatory and anti-inflammatory cytokines following oral administration. In this research, consisting of three separate studies, NEM® was administered daily at doses of 6.13 mg/kg bw/day (Study 1), 10.0 mg/kg bw/day (Study 2), or at doses of 0 (control), 26.0, or 52.0 mg/kg bw/day (Study 3) by oral gavage for 7 consecutive days. These doses, following allometric conversion, equate to a human equivalent dose (HED) of 59 mg/day, 97 mg/day, 252 mg/day and 503 mg/day respectively. (A 500 mg/day dose has been shown in clinical trials to be the optimal effective dose of NEM®.) Healthy rats were evaluated in Study 1 and Study 2, whereas inflammation was induced in the Study 3 rats by intraperitoneal injection of lipopolysaccharide. Changes in the levels of a broad range of both pro-inflammatory and anti-inflammatory cytokines and chemokines were analyzed.

The two primary mediators (cytokines) of joint inflammation are IL-1β and TNF-α. These cytokines can in turn induce chondrocytes (cartilage cells) to produce matrix metalloproteinases (MMPs), chemokines (IL-8, MCP-1, MIP-1α, MIP-1β, RANTES and others), as well as nitric oxide and prostaglandins, which subsequently leads to localized tissue destruction, immune cell infiltration, inhibition of cartilage matrix synthesis, and increased pain sensitivity.

Even though the studies in healthy rats incorporated low doses of NEM®, the results revealed a trend towards reduction of both IL-1β (Study 1) and TNF-α (Study 2) and statistically significant effects for nearly all of the pro-inflammatory chemokines (MCP-1, MIP-1α, MIP-1β, RANTES, VEGF) that are currently understood to play key roles in the inflammation and pathogenesis associated with both osteoarthritis and rheumatoid arthritis. It is noteworthy that there was no negative effect from oral supplementation with NEM® on anti-inflammatory cytokines and chemokines (L-4, IL-6, IL-10 and TIMP-1) in the healthy rats.

In inflammation-challenged rats (Study 3), there was a substantial (39 to 44 percent) and lasting (through 24 hours) reduction in IL-1β and a smaller but substantial (19 to 32 percent) decrease in TNF-α levels as well. These effects on the key mediators of joint inflammation provide further supportive evidence to the observed clinical efficacy of NEM®.

Evaluation of the results from all three studies demonstrated that oral supplementation with NEM® can influence both early-phase pro-inflammatory cytokines like IL-1β and TNF-α when inflammation is present. NEM® can also influence later-phase pro-inflammatory cytokines like MCP-1, MIP-1α, MIP-1β, RANTES and VEGF under healthy non-inflammatory conditions. These results not only corroborate the results from the in vitro mechanism of action study, but also point toward an exceptionally safe intervention for inflammatory conditions.