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FILE: ▪ Red Clover (*Trifolium pratense*)
▪ Isoflavones
▪ Inflammation

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RE: In vitro Anti-inflammatory Effects of Red Clover Isoflavones

Lam ANC, Demasi M, James MJ, Husband AJ, Walker C. Effect of red clover isoflavones on Cox-2 activity in murine and human monocyte/macrophage cells. *Nutr Cancer.* 2004;49(1):89–93.

Red clover (*Trifolium pratense*) is a perennial herb native to Europe, central Asia, and northern Africa. It is naturalized to North America. Red clover has traditionally been used for many different ailments, including diarrhea, upper respiratory tract infections, psoriasis, and eczema. Red clover is a good source of calcium, magnesium, potassium, chromium, zinc, niacin, thiamine, and vitamin C. Researchers have studied the isoflavones formononetin, genistein, daidzein, and biochanin found in high concentrations in red clover and soy (*Glycine max*) for their abilities to decrease perimenopausal hot flashes and to prevent postmenopausal bone loss. The anti-inflammatory effects of isoflavones were explored in this placebo-controlled, in vitro study.

Mouse macrophage cell line RAW264.7 and human monocytes were incubated separately in lipopolysaccharides (LPS) to induce inflammation. LPS is a component of bacterial cell walls that are capable of producing strong immune reactions. The cells were then treated with genistein, biochanin, daidzein, and formononetin to determine their abilities to decrease inflammatory metabolites. Prostaglandin E_2 (PGE_2) production was tested in mouse macrophage cells and PGE_2 plus thromboxane B_2 (TXB_2) were tested in human monocytes. PGE_2 stimulates inflammation, and TXB_2 stimulates blood clotting.

All four isoflavones significantly inhibited PGE_2 and TXB_2 compared to control. Genistein was the strongest inhibitor of PGE_2 in murine macrophages. Compared to control, it significantly decreased PGE_2 by 62% at a concentration of 1mcM ($P < 0.001$). Formononetin and biochanin significantly decreased PGE_2 compared to control by 60% and 75% at 10 mcM, respectively ($P < 0.001$), while daidzein significantly inhibited PGE_2 compared to control by 32% at 40 mcM ($P < 0.001$).

Production of PGE_2 by human monocytes was inhibited by genistein and formononetin at 10 mcM ($P < 0.001$). Those two, plus daidzein, inhibited PGE_2 at 10 mcM ($P < 0.001$). Only formononetin significantly inhibited TXB_2 at 10 mcM ($P < 0.05$). But formononetin, genistein, and biochanin decreased TXB_2 at 100 mcM ($P < 0.001$). Cells remained viable even after being treated with the highest dosage of each drug in each cell line.

PGE_2 and TXB_2 are catalyzed by the cyclooxygenase (COX) enzyme, and their elevation is seen in many diseases, including arthritis and cancer. Researchers are searching for methods of inhibiting COX that do not have the toxicity associated with non-steroidal anti-inflammatory drugs (NSAID) such as ibuprofen and aspirin. While the concentrations of the isoflavones tested in this study are within those obtainable by ingestion, in vivo studies need to be conducted to confirm these results and test their clinical relevance.

—John Neustadt, ND

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