# Synergistic Beneficial Effects of Curcuma Extract, Green Tea Extract and Hydrolyzed Collagen in Bovine Chondrocytes in Monolayer Culture

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**INTRODUCTION** Osteoarthritis (OA) is a chronic, painful and inflammatory condition that affects the joints and leads finally to the loss of mobility. The most popular treatments are non steroidal anti-inflammatory drugs but these are associated with adverse side effects, especially when long-term used. A safer treatment, which could come from nutraceuticals, would be desirable. This study aimed to investigate the effects of curcuma extract, hydrolyzed collagen and green tea extract, alone or in combination, on the production and gene expression of inflammatory and catabolic mediators by primary bovine chondrocytes.

**METHODS** Bovine chondrocytes were isolated from normal bovine articular cartilage and cultured in monolayer until confluence. Chondrocytes were then incubated with or without 12.5 µg/ml curcuma extract/hydrolyzed collagen/green tea extract, alone or in combination, and in the absence or in the presence of $10^{-10}$M interleukin-1β (IL-1β). After 24 hours of incubation, interleukin-6 (IL-6), inducible nitric oxyde synthase (iNOS), cyclooxygenase 2 (COX-2), metalloproteinase 3 (MMP-3), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and -5 expressions were determined by real time PCR. After 48 hours of incubation, nitric oxide (NO) and prostaglandin $E_2$ ($PGE_2$) productions were quantified.

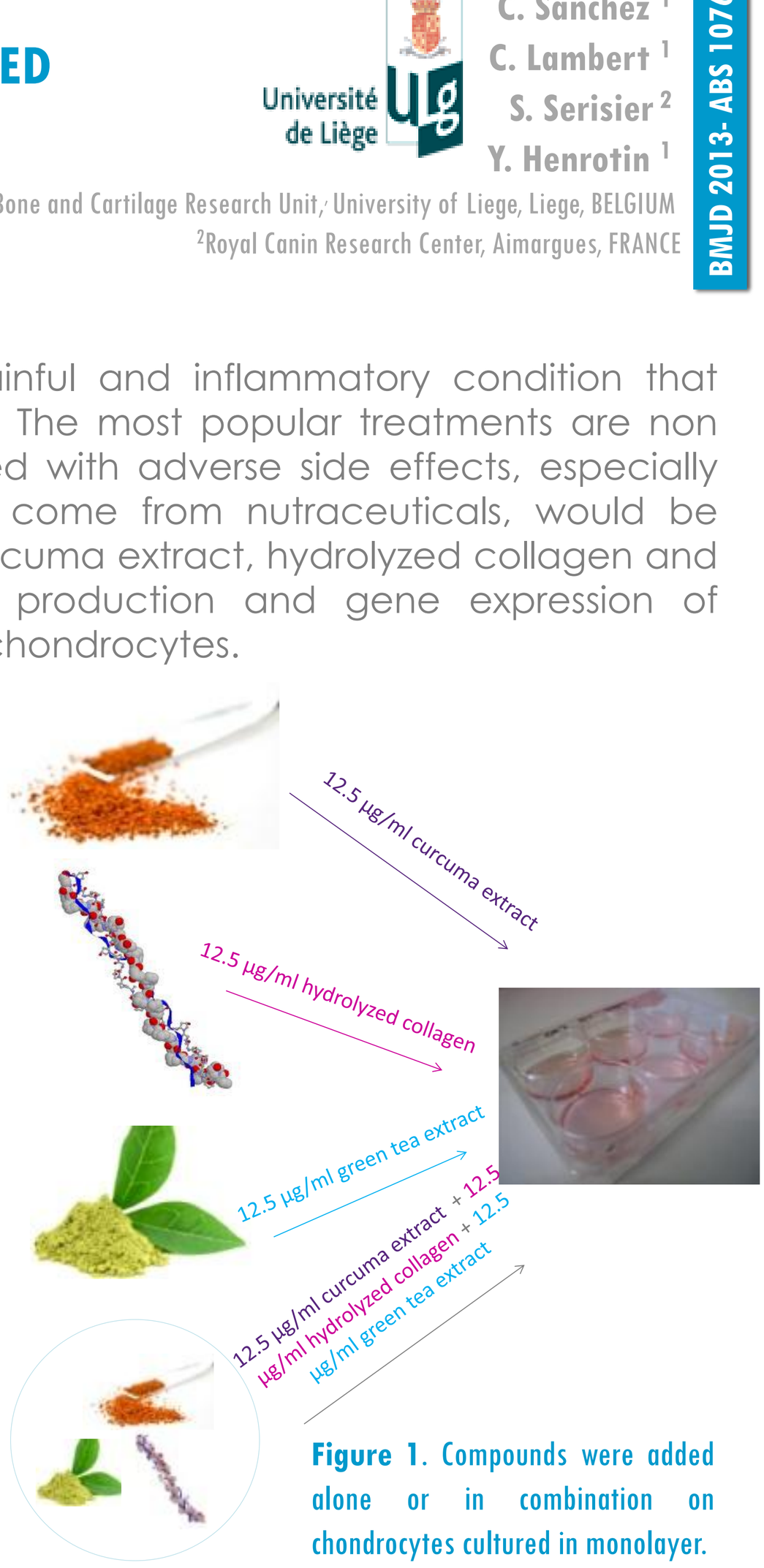


**Figure 1**. Compounds were added alone or in combination on chondrocytes cultured in monolayer.

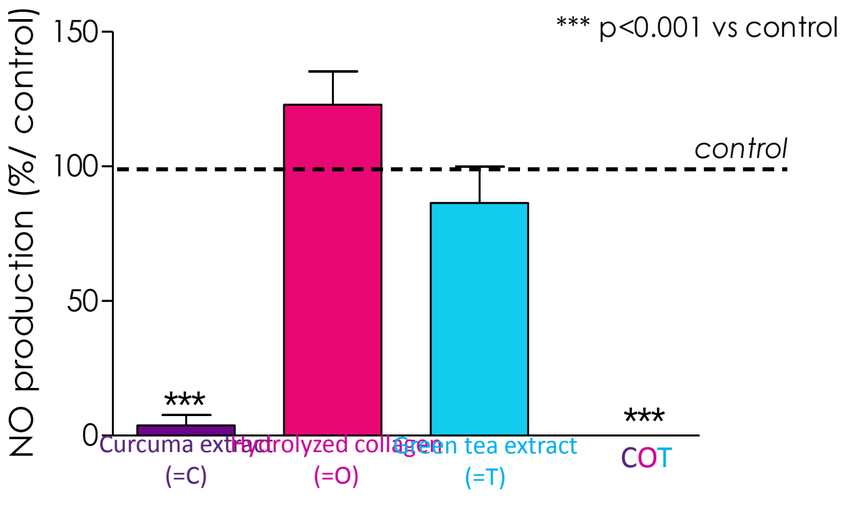


**Figure 2**. Curcuma extract alone and the mixture of the three compounds curcuma extract/hydrolyzed collagen/green tea extract (COT) significantly inhibited NO production ($p<0.001$).

**RESULTS** Curcuma extract alone significantly inhibited IL-1β stimulated NO production and IL-1β stimulated IL-6, iNOS, MMP-3 and ADAMTS4 expressions. Hydrolyzed collagen and green tea extract alone did not significantly inhibit inflammatory and catabolic mediators synthesis. The combination of the three compounds significantly inhibited IL-1β stimulated NO production and IL-1β stimulated IL-6, iNOS, MMP-3 and ADAMTS4 expressions. Moreover, the mixture significantly inhibited COX-2 and ADAMTS5 expressions whereas the compounds alone had no significant effect. When they were combined, curcuma extract, hydrolyzed collagen and green tea extract had a synergistic effect on the inhibition of NO production and IL-6 and iNOS expressions, compared to the effect of each compound alone.

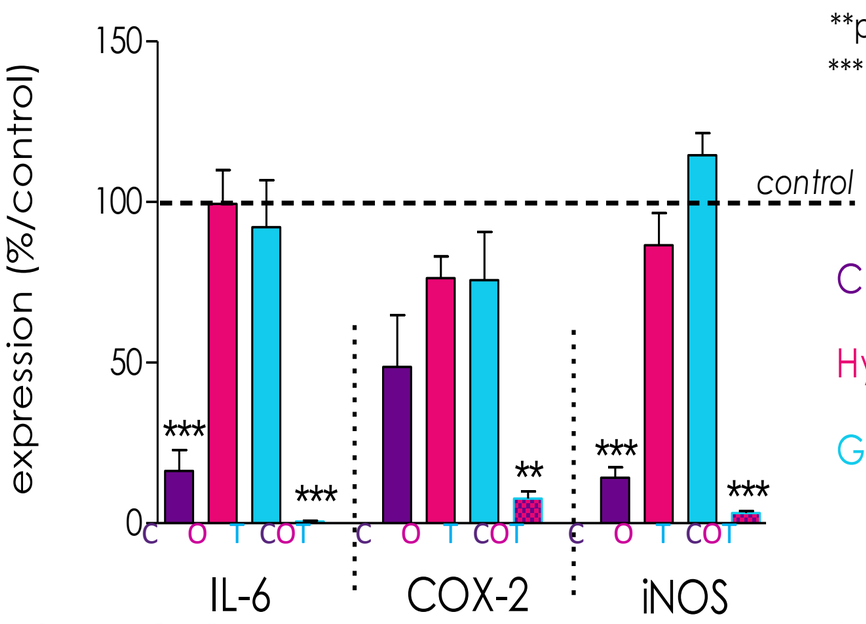


**Figure 3**. Curcuma extract alone significantly inhibited IL-6 and iNOS expressions ($p<0.001$). The mixture of the three compounds curcuma extract/hydrolyzed collagen/green tea extract (COT) significantly inhibited IL-6, COX-2 and iNOS expressions ($p<0.01$). For IL-6 and iNOS expressions, the effect of the mixture was better than expected.

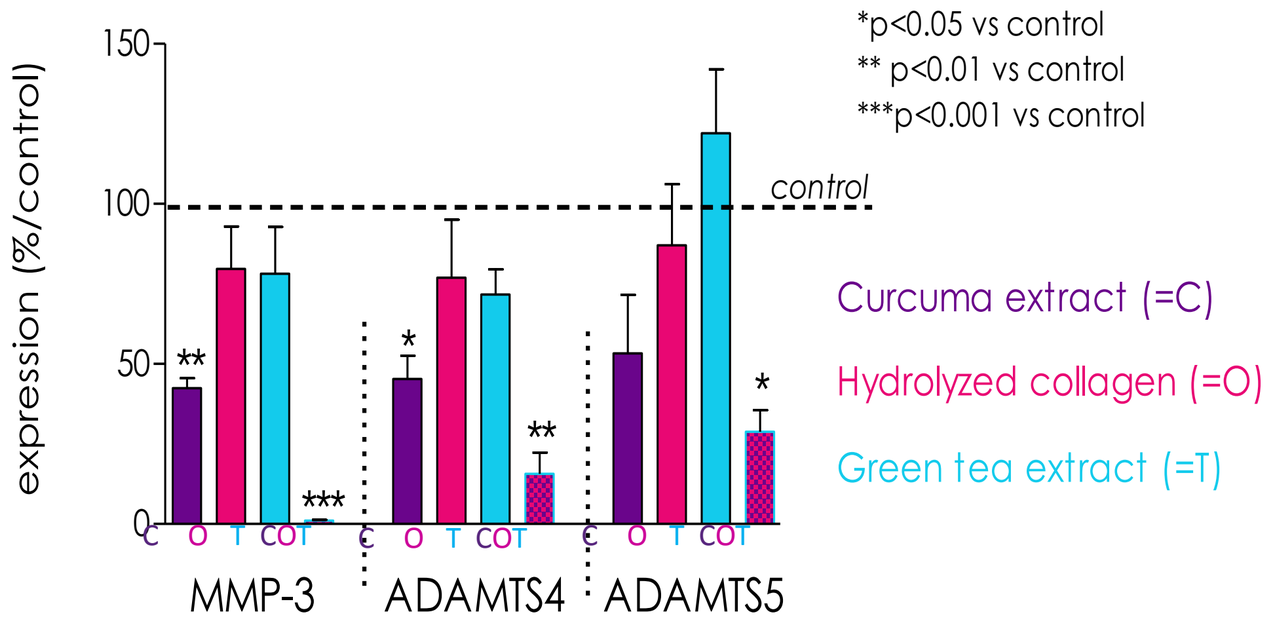


**Figure 4**. Curcuma extract alone significantly inhibited MMP-3 and ADAMTS4 expressions ($p<0.05$). The mixture of the three compounds curcuma extract/hydrolyzed collagen/green tea extract (COT) significantly inhibited MMP-3, ADAMTS4 and ADAMTS5 expressions ($p<0.05$).

**CONCLUSION** The combination of the three compounds curcuma extract/hydrolyzed collagen/green tea extract showed beneficial effects on the production of inflammatory mediators and on the expression of genes involved in catabolism and inflammation. Moreover, these in vitro results indicated that curcuma extract, hydrolyzed collagen and green tea extract acted synergically to inhibit the production of inflammatory mediators and the expression of genes involved in inflammation. These findings provide a preclinical basis for the in vivo testing of this combination.