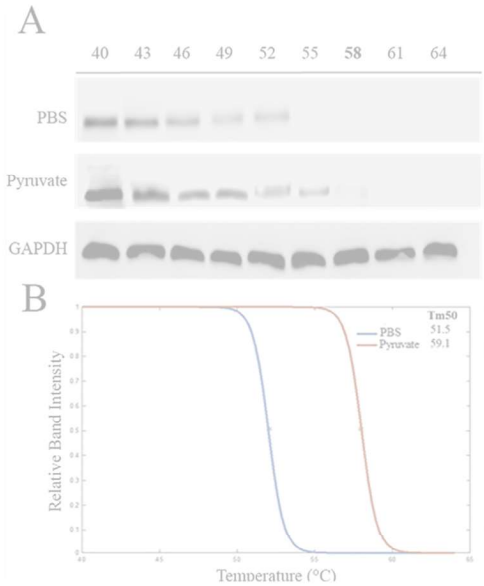

Figure 3: (A) cellular thermal shift assay (CETSA) followed by western blot to confirm binding target. different conditions (B) CETSA melt response and associated curve.

PRESENTATION NUMBER: 432
BIOLOGICAL EFFECT OF APPA -APOCYNIN AND PAEONOL- IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: Osteoarthritis (OA) is the most common rheumatological disease and a major cause of pain and disability in older adults. Currently no treatment is available that alters the course of OA. Mitochondrial dysfunction in chondrocytes is associated with the molecular dysregulation underlying OA, and has been proposed as a potential therapeutic target. APPA, a combination of apocynin (AP) and paeonol (PA), has the potential capacity to protect mitochondria from injury. ***Aim*** To study the biological effect of APPA in human articular chondrocytes.

Methods: Tissue and chondrocytes from human OA cartilage were isolated. The effect of APPA on chondrocyte viability was analyzed using MTT. ROS production (cytoplasmic and mitochondrial) was evaluated by flow cytometry using DCFH-DA and MitoSoxRed®. The effect of APPA on expression of pro-inflammatory cytokines genes (*IL-6*, *IL-8* and *TNF-α*, *MMP-13* and *MMP-3*) was analyzed in chondrocytes and cartilage by RT-PCR. Quantification of Toluidine Blue (TB) staining in cartilage was performed to evaluate proteoglycans content. Appropriate statistical analyses were performed with GraphPad Prism v6.

Results: Chondrocytes incubated in the presence of APPA 10 and 20 μg/mL for 24 h had greater than 85% viability. APPA 10 μg/ml reduced the cytoplasmic ROS production induced by LPS (28.56 %, $p \leq 0.005$). APPA significantly reduced the gene expression induced by IL-1β 10 ng/mg of *IL-8*, *TNF-α*, *MMP-13* and *MMP-3* (**Table-1**). In cartilage, incubation with APPA 100 μg/ml for 24 h and 48 h decreased the *MMP-3* gene expression induced by *IL-1β* (51.63±25.71 and 8.88±2.94 respectively) (**Figure-1**). Quantification by TB showed that APPA at 100 μg/ml increased the proteoglycans in the intermedial layer (227,8±67) **(Figure-2)**.

Conclusions: On human articular chondrocytes, APPA has anti-inflammatory effect and could reduce extracellular matrix degradation of cartilage. Effects of APPA could be mediated by its capacity to modulate ROS production. APPA has the potential to affect the progression of joint damage in OA

Gene expression of pro-inflammatory cytokines.

	IL-1β+APPA-10 μg/ml	p value
IL-6	87.14±17.23	0.495
IL-8	52.68±14.27	0.008
TNF-α	55.12±12.08	0.002
MMP-13	69.37±7.79	0.002
MMP-3	72.29±9.42	0.03

Table 1 Effect of 10 μg/ml APPA on mRNA expression IL-6, IL-8, TNF-α, MMP-13, MMP-3, All data were obtained from six independent donors, each with two replicates. Values are presented as mean ± SEM relative to IL-1β (as 100%).

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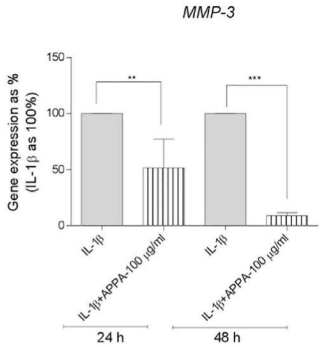
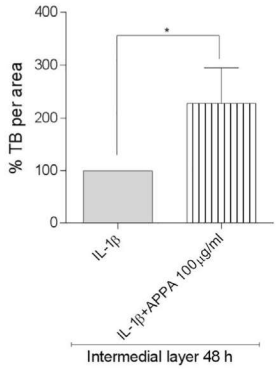


Time	IL-1β+APPA-100 μg/ml	p value
24 h	51.63±25.74	0.001
48 h	8.883±2.94	≤0.0001

Figure 1. Effect of APPA on mRNA expression *MMP-3*. All data were obtained from five independent donors, each with two replicates. Values are presented as mean ± SEM relative to IL-1β (as 100%).



	IL-1β+APPA 100 μg/ml	p value
48 h	227,8±67.4	0.018

Figure 2. Effect of APPA on the proteoglycans content. Quantificacion of Toloudin Blue (TB) in the intermedial layer of the cartilage. All data were obtained from five independent donors, each with two replicates. Values are presented as mean ± SEM relative to IL-1β (as 100%)

PRESENTATION NUMBER: 433
MODULATION OF JOINT INFLAMMATION USING TRIAMCINOLONE ACETONIDE LEADS TO SUSTAINED MACROPHAGE INFLAMMATORY RESPONSES AND ENHANCED OSTEOPHYTE FORMATION DURING EXPERIMENTAL OSTEOARTHRITIS IN MICE

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Purpose: Joint inflammation is a prominent feature of osteoarthritis (OA) pathogenesis associated with pain and disease progression, with