Introduction

- **Osteoarthritis**
  - Global age-standardized prevalence of knee OA is 3.8% and hip OA is 0.85%, with no major changes from 1990 to 2010.
  - Recent study have focused on the mechanism and concomitant therapy of OA.
  - They emphasized that the importance of the molecular changes such as inflammation and oxidative stress.

- Many studies have proven that anthocyanins species such as delphinidin suppress the progression of OA through its antioxidant capacity via various pathways.
  - Furthermore, recent studies concerning the mechanisms of the development of various degenerative diseases revealed that delphinidin is correlated to apoptosis or autophagy.
  - However, very few studies have been found to know exactly what mechanisms
    - apoptosis or autophagy, are involved between the development of OA and delphinidin.

**Classification of cell death mechanism**

- spontaneous cell death by degeneration
- Programmed cell death
- Type I programmed cell death (apoptosis)
- Type II programmed cell death (autophagy)

Materials and methods

- **Cell and chemicals**
  - The human C28-12 chondrocytes cells were prepared and cultured in Dulbecco Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12).

- **Determination of cell viability**
  - The effect of delphinidin on cell viability was determined by CCK-8 assay and it was assessed as percent cell viability, where vehicle-treated cells were considered as 100% viable.

- **Trituration of delphinidin for cytotoxicity in C28-12 chondrocyte cells**
  - The C28-12 cells were treated with different concentration of delphinidin (10-75 µM) for 2 hours, 4 hours and 24 hours.

- **Western blotting and Flow cytometry**
  - To validate whether delphinidin can promote autophagy activation, we also observed autophagosome formation using western blotting analysis.
  - **DAPI staining**
    - DAPI (4', 6-diamidino-2-phenylindole) staining for evaluation of DNA fragmentation was performed.
  - **TUNEL assay**
    - Terminal deoxynucleotidyl transferase (TDT)-mediated dUTP nickend labeling (TUNEL) assay was used as a measure of apoptosis.

Results

- **Hydrogen peroxide induced C28-12 Chondrocytes cells death**
- **Trituration of delphinidin for cytotoxicity in C28-12 cells**

- **Delphinidin protects C28-12 Chondrocytes cells in hydrogen peroxide cytotoxicity**

- **Delphinidin protect C28-12 cells from H2O2 induced oxidative stress via NRF2 and NF-κB**

- **Delphinidin induced autophagy and protect C28-12 cells from H2O2 induced oxidative stress via NRF2 and NF-κB**

- **Inhibition of delphinidin induced autophagy increase H2O2 induced apoptosis in C28-12 cells**

Discussions

- When we analyzed the effect of delphinidin on oxidative stress in chondrocyte using rapamycin which acts as autophagy inducer and chloroquine which acts as autophagy inhibitor, we observed the decrease in caspase-3, PARP and the increase in Bcl-XL, NF-κB, and NF-kB.
- These results mean that we proved delphinidin causes apoptosis in oxidative stress chondrocyte, and that delphinidin-induced autophagy also inhibits apoptosis, thus, it have cytoprotective roles in oxidative stress chondrocyte.

Conclusions

- **Delphinidin compromise cellular protective mechanisms, inhibiting apoptosis to cell death and finally cause autophagy cell protection.”**

- **Ten tailed Student’s t-test. One-way analysis of variance statistics was used to compare the means of three groups or more, followed by a Tukey’s multiple comparison test.”**