GROUPE GRAP'SUD
L'autre richesse du raisin

BREKOO

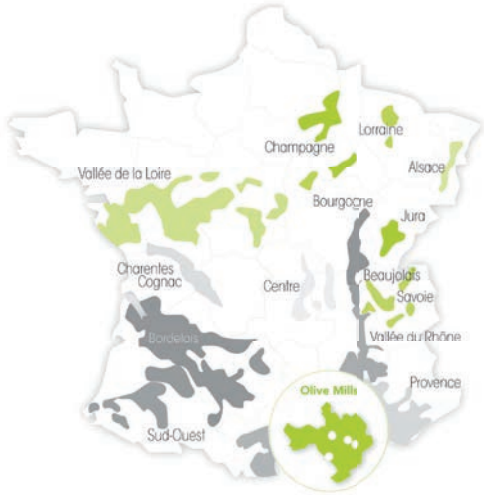
Healthy aging

oléoGrape® SEED

OleoGrape®SEED is a natural standardized polyphenols extract made from selected French olives and grapes, with guaranteed contents in active components:

- **Total Polyphenols: ≥ 95 %**
- **Procyanidins (OPC): ≥ 30 %**
- **Hydroxytyrosol: ≥ 6 %**

OleoGrape®SEED has been developed by GRAP'SUD, a French company located at the heart of Occitanie and Provence vineyards, whose specialization is the extraction of grape and olive polyphenols.



BENEFITS

According to the World Health Organization[1], the proportion of the world's population over 60 years will nearly double from 12% to 22% between 2015 and 2050, as a result of both longer life expectancy and declining fertility rates. This population embodies a big society challenge in order to maximize the health and functional capacity of older people as well as their social participation and security. It is a **major public health issue** for many countries to increase the healthy living time and reduce the morbidity which represents also a high **cost for the societies**.

The transition from high to low mortality and fertility has also meant a shift in the leading causes of disease and death, called "epidemiologic transition". It is characterized by the emerging importance of chronic and degenerative diseases: **cardiovascular disease, cancers, or dementia** affect particularly the elderly. **Osteoarthritis** is aligned with these consideration. This common inflammatory joint disease affects a growing part of the elderly and is associated with a strong socio-economic impact.

Many studies have shown beneficial effects of phenolic compounds from olive and grape seeds on health, partly due to their chemical structure with many phenol cycles and hydroxyl groups. Hydroxytyrosol has powerful antioxidant[2] and anti-inflammatory properties[3]. Procyanidins have antioxidant activity and the ability to inhibit the synthesis of numerous inflammatory mediators[4]. **Osteoarthritis** is characterized by progressive cartilage loss, subchondral bone remodeling, osteophyte formation, and joint tissues inflammation. Considering their anti-inflammatory and anti-oxydative properties, procyanidins and hydroxytyrosol are good candidates to prevent osteoarthritis. As part of an **on-going research and development program initiated since 2010 by GRAP'SUD**, OleoGrape®SEED properties have been tested in vitro and in animals in collaboration with the "Skeletal Tissue Engineering and Physiopathology" team of the French National Institute of Health and Medical Research (Inserm). The program was supported by the French National Research Agency, the Occitanie region and BPIFrance.

OleoGrape®SEED shows interesting results on prevention of osteoarthritis and significant effects on depigmenting. Bioavailability and bioactivity of the extract were also proven. Some of these promising results were published in peer-reviewed scientific journals.

APPLICATIONS

- **FOOD SUPPLEMENT**
- **COSMETICS**
- **FUNCTIONAL FOODS**

OSTEOARTHRITIS: IS THE NUTRITIONAL PREVENTION A SOLUTION?

The Osteoarthritis research society international (OARSI) defined osteoarthritis as "a disorder involving movable joints characterized by cell stress and extracellular matrix degradation initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic, and/or physiologic derangements (characterized by cartilage degradation, bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness"[5] **(Figure 1).**

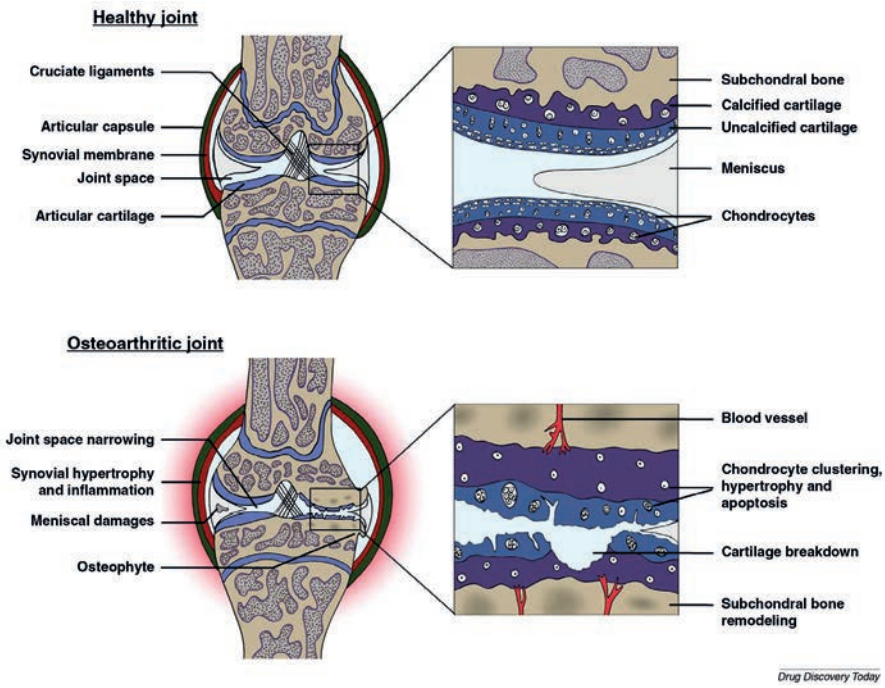


Figure 1: Joint tissue affections in knee osteoarthritis[6].

In osteoarthritis, cartilage degradation is observed and a vicious cycle of inflammation sets in with alteration of the habitual mechanism of joint cells. Markers of this inflammation are the **inflammatory cytokines such as interleukin-1 beta** (IL-1ß), which stimulates the synthesis of **nitric oxide (NO), prostaglandin E_2 (PGE_2) and matrix metalloproteinases (MMPs)**. The cartilaginous extracellular matrix (ECM) is weakened by the breakdown of some of its constituents, type II collagen and aggrecan, linked to MMP-13 effect[7].

As a result, cytokines and MMPs are secreted into the synovial fluid[8] and the loop of inflammation is intensified. For these reasons, **inflammation and catabolism of extracellular matrix represent key events that can be targeted for the identification of novel therapeutic interventions in osteoarthritis[9].**

The OsteoArthritis Research Society International (OARSI) scoring system can be used for histological assessment[10]: the severity of OA lesions is scored on a scale ranging from 0 to 5 using parameters such as chondrocyte death, hypertrophy, clusters, loss of Safranin-O staining, surface alteration, and bone modifications (total maximum score 25).

To date, there is no curative treatment for osteoarthritis in humans. Therefore, clinical management focuses only on osteoarthritis symptoms (e.g. pain and inflammation), using analgesics and nonsteroidal anti-inflammatory drugs. **Nutritional prevention could be another approach of the osteoarthritis.**

ANTI-IL-1ß EFFECT OF OLEOGRAPE®SEED PRE-TREATMENT IN RABBIT ARTICULAR CHONDROCYTES[9]

This study evaluated whether OleoGrape®SEED could have anti-inflammatory and chondroprotective actions. These actions were tested in vitro on primary cultured rabbit articular chondrocytes (RAC) stimulated by interleukin-1 beta (IL-1ß).

The effects on the expression of iNOS, COX2 and MMP13 transcripts were determined as well as associated products NO, PGE2 and MMP-13 were investigated.

Results showed an increased expression of iNOS, COX2 and MMP13 in response to IL-1ß treatment **(Figure 2: A-C)**. In the OleoGrape®SEED condition, results demonstrated a significant decrease in IL-1ß -induced iNOS, COX2 and MMP13 expression levels. **Consistent with gene expression levels, the IL-1ß -dependent NO, PGE2 and MMP-13 productions were also significantly decreased by the extracts in RAC** (**Figure 2: D–F)** and the IL-1ß-induced MMP-13 production was even totally suppressed **(Figure 2F)**.

Author, year: Mevel et *al.*, 2016
Design: Cells pre-incubated for 24h in the presence of OleoGrape®SEED. Human recombinant IL-1ß (1ng/mL) then added for an additional 24h. Real-time polymerase chain reaction (RT-PCR) was performed and ß-actin was used as reference gene to express results as relative expression levels.
Dose: OleoGrape®SEED at a concentration of 10mg/L

Figure 2: In vitro OleoGrape®SEED effects on iNOS, COX2 and MMP13 expression and NO, PGE2 and MMP-13
The relative expressions of iNOS (A), COX2 (B) and MMP13 (C) compared to ß -actin were evaluated by real time RT-PCR.
NO production (D) was assessed using Greiss reaction, PGE2 (E) and MMP-13 (F) productions were measured using ELISA assay.
#$p < 0.05$ compared to the condition with IL-1ß (1 ng/mL) alone, *$p < 0.05$ compared to the condition without IL-1ß with the same extract solution.

PREVENTIVE EFFECT OF OLEOGRAPE®SEED ON POST-TRAUMATIC OSTEOARTHRITIS ANIMAL MODEL[9]

Author, year: Mevel et *al.*, 2016
Design: 15 week-old female white rabbits which underwent osteoarthritis-induced surgery. The severity of osteoarthritis was assessed by X-ray evaluation and OARSI scoring was assessed on histological stainings.
Intervention: Rabbits underwent a destabilization of the right joint induced by anterior cruciate ligament transection (ACLT).
Dose: 100mg/kg of glucosamine (GlcN) or OleoGrape®SEED every two days
Duration: osteoarthritis-induced surgery 3 weeks after start of supplementation, euthanasia 10 weeks after the surgery

The objective of the study was to determine whether the diet supplementation with OleoGrape®SEED could exert anti-osteoarthritis effects in model of post-traumatic osteoarthritis in rabbit. They compared effects of OleoGrape®SEED to glucosamine, commonly used as slow-acting anti-arthritic.

The results shown no joint alteration in non-operated knees (control). In contrast, ACLT knees from rabbits fed with the standard diet (standard group) present osteoarthritis pathological changes characterized by increased ligament calcification, osteophytes incidence and bone sclerosis **(Figure 3A)**. **Both glucosamine and OleoGrape®SEED intake decreased the lesion severity.**

Radiographic OA scoring revealed a significant decrease in the two supplemented groups compared to the standard group (Figure 3B).

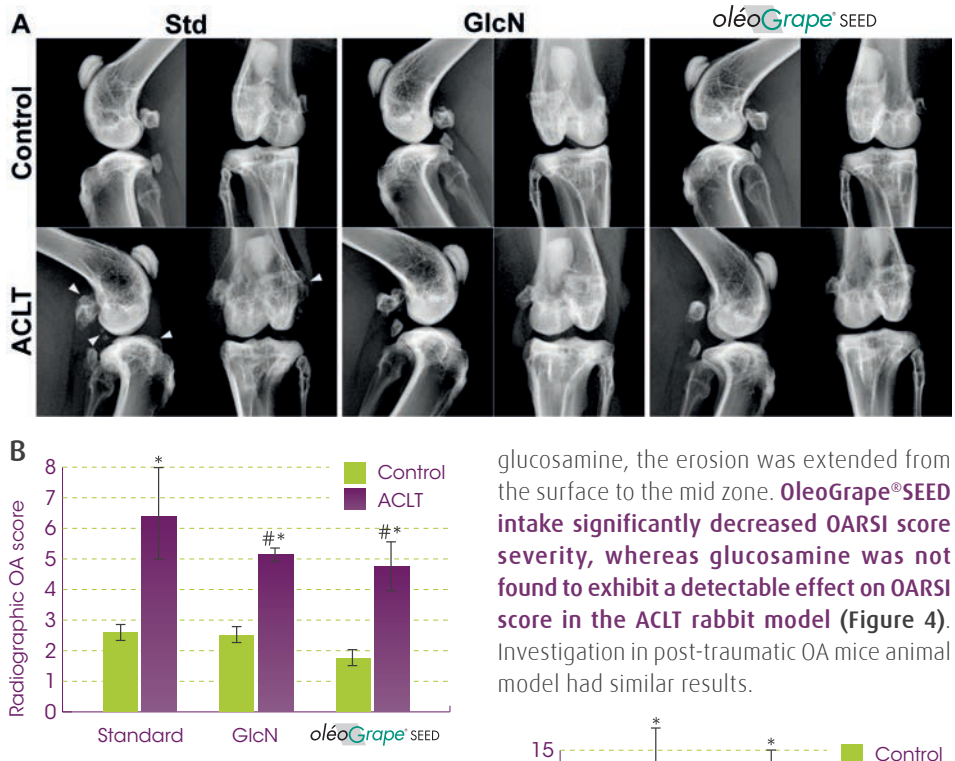


Figure 3: OleoGrape®SEED decreased radiographic OA score on ACLT rabbit model.
X-ray (A) and corresponding radiographic score (B) of non-operated knee (control) or operated knee (ACLT) untreated rabbits or treated with GlcN or OleoGrape®SEED at 10 weeks subsequence to surgery. *p > 0.05 compared to non-operated knee (control) within the same group; #p > 0.05 compared to ACLT receiving NaCl (Std) group. Arrowheads show osteophytes formation after ACLT.

The histological staining in experimental ACLT group treated with OleoGrape®SEED did not show any sign of cartilage erosion, but only presented cartilage swelling (oedema). On the opposite, in ACLT rabbits treated with glucosamine, the erosion was extended from the surface to the mid zone. **OleoGrape®SEED intake significantly decreased OARSI score severity, whereas glucosamine was not found to exhibit a detectable effect on OARSI score in the ACLT rabbit model (Figure 4)**. Investigation in post-traumatic OA mice animal model had similar results.

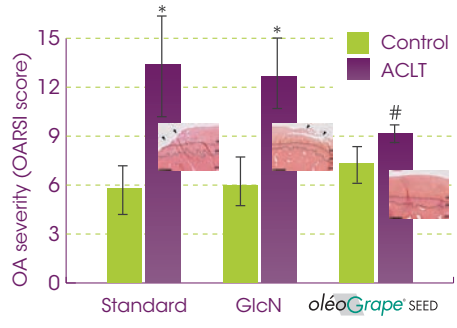
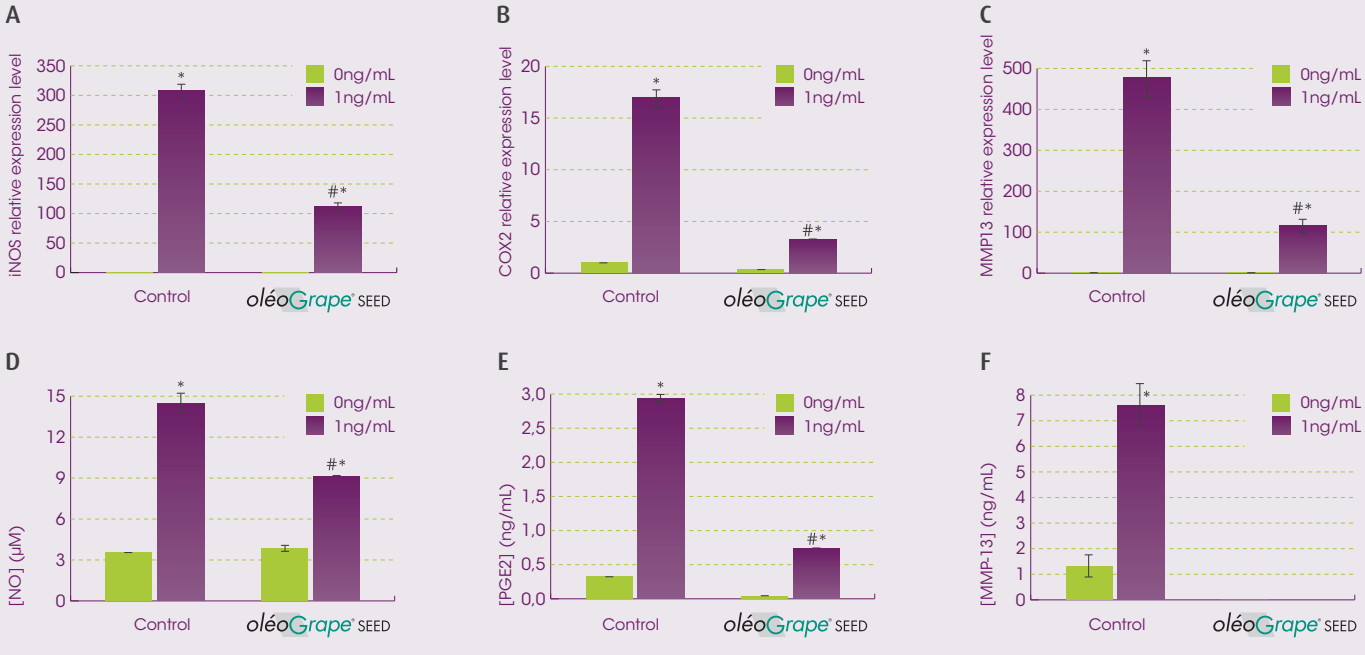


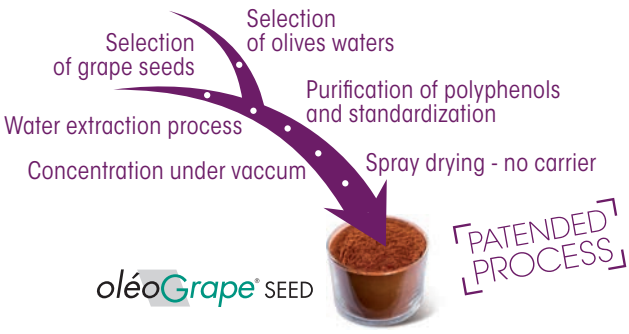
Figure 4: HES staining of cartilage (A) and OARSI scores (B) of non-operated knee (control) or operated knee (ACLT) at 10 weeks post-surgery. *p > 0.05 compared to non-operated knee (control) within the same group; #p > 0.05 compared to ACLT receiving NaCl (Std) group



TECHNICAL INFORMATION

OleoGrape®SEED is a natural standardized polyphenols extract made from selected grapes *(Vitis vinifera)* and olive *(Olea Europea)* harvested exclusively in France. It offers guaranteed contents in procyanidins, and hydroxytyrosol.

*GRAP'SUD holds a patent with CIRAD and SUPAGRO on OleoGrape®SEED fabrication process, called "Extraction process of phenolic compounds from olive vegetable water and polyphenols titrated extract preparation from olive and grape" (filing number: 09/00752, filing date: 02/08/2009).



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WHITENING EFFECT OF OLEOGRAPE®SEED[11]

Author, year: BIOalternatives, 2008
Design: Culture of murine melanocytes during 72h at 37°C, 5%CO2
Dose: Kojic acid from 25 to à 800 µg/mL (used as reference), OleoGrape®SEED at 12, 37 and 110 µg/mL.

Skin changes are among the most visible signs of aging. The outer skin layer (epidermis) thins, the number of melanocytes, the pigment-containing cells, decreases and the remaining melanocytes increase in size. Consequently, aging skin looks thinner, paler, and clear (translucent). Large pigmented spots, including age spots, liver spots, or lentigos, may appear in sun-exposed areas[12]. The **MSH hormone** (Melanocyte-Stimulating Hormone) is responsible of tanning by stimulating melanin synthesis by melanocytes.

This study evaluated the depigmenting effect of OleoGrape®SEED extract on melanocytes. The cells were cultured without or with stable derivative of α-MSH (NDP-MSH) and without (control) or with test compound: kojic acid, which is an inhibitor of melanin production, used as reference, or OleoGrape®SEED extract. Inhibition percentage was calculated as followed:

Inhibition % =
100 – (Value/Control mean) x 100

Results showed that **OleoGrape®SEED has a whitening concentration-depending effect, inhibiting melanin synthesis by cultivated melanocytes, with or without α-MSH**. None of the OleoGrape®SEED concentration altered cell viability.

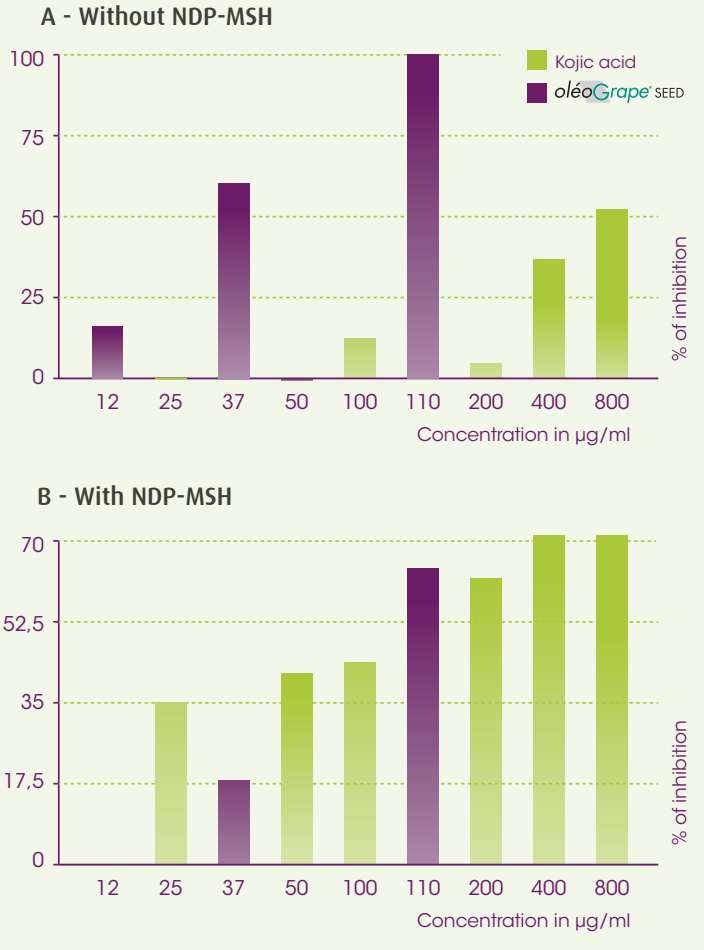


Figure 7: Inhibition percentage of melanin production, without MSH hormone (A) or with MSH hormone (B)

BIOAVAILABILITY AND BIOACTIVITY OF OLEOGRAPE®SEED AFTER ORAL INTAKE[9]

Author, year: Mevel et *al.*, 2016
Design: 15 week-old female white rabbits were randomly divided into 2 groups (n = 4 per group): control group fed with saline solution (1 mL/kg) and experimental group with 6 doses of OleoGrape®SEED for 8 days. Venous blood was collected, centrifuged and sera were stored and frozen at – 80 °C until their use in cell culture and Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) experiments.
Dose: OleoGrape®SEED was administrated by gavage at the dose of 100 mg/kg (for cell culture) or 500 mg/kg (for UPLC-MS analysis) in saline solution.

Biological properties of phenolic compounds depend on bioavailability (capacity to go in blood circulation or organs after consumption). After ingestion, phenolic compounds undergo several transformation steps: absorption by intestinal cells, intestinal or hepatic metabolism and excretion by urinary or biliary tract. Procyanidins have polymeric structure and high molecular weight which limit intestinal absorption. However only few studies brought results on in vivo absorption of PCy, with divergent conclusions. Also few studies investigated olive compounds bioavailability, and particularly hydroxytyrosol. HT is well absorbed through epithelial cells of gastrointestinal tract, where it is biotransformed. Bioavailability of the native form is however very low. The authors investigated the bioavailability and bioactivity of OleoGrape®SEED extract, following gavage with the extract in rabbits.

First they explored whether the sera of rabbits may contain the regular metabolites of phenolic compounds. **Five metabolites from HT and nine from PCy were found (Figure 5)**. None of these metabolites were found in the serum of rabbit fed with NaCl.

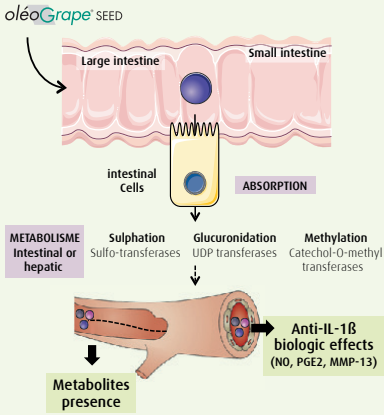
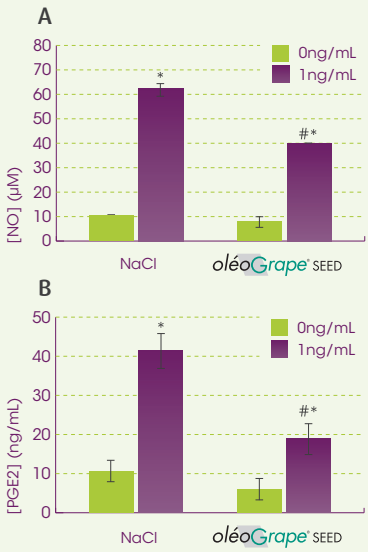


Figure 5: Bioavailability of OleoGrape®SEED following gavage in rabbits: Absorption, metabolism and excretion of polyphenols

Then they investigated the maintenance of the anti-IL-1ß effects of sera from rabbits after oral intake of OleoGrape®SEED. Results are shown in the **figure 6**.
The authors concluded that the sera of rabbit fed with OleoGrape®SEED contained metabolites with anti-IL-1ß biological effects. Investigation of bioavailability and bioactivity following gavage of dogs had similar results (unpublished results).



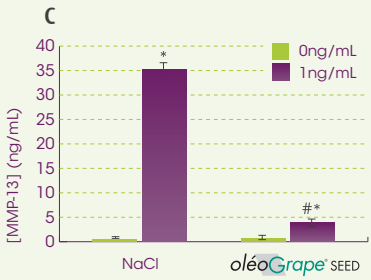


Figure 6: Effects of serum from rabbit fed with NaCl or OleoGrape®SEED on the IL-1ß-induced levels of NO (A), PGE2 (B) and MMP-13 (C) production.
#p < 0.05 compared to the NaCl condition with IL-1ß (1 ng/mL),
*p < 0.05 compared to the condition without IL-1ß with the same extract solution.