Modulatory effects of a lunasin-enriched soybean extract on immune response and oxidative stress-associated biomarkers

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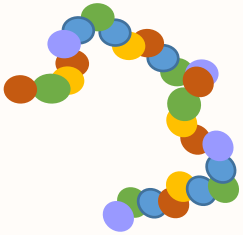
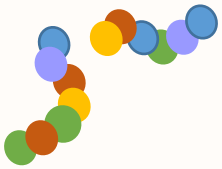
Introduction

Bioactive Peptides



Microbial fermentation
Chemical or enzymatic hydrolysis

Digestion or industrial processing



Precursor protein

Animal Origin

Milk
Eggs
Meat

Plant Origin

Cereals
Legumes
(Soybean)

- Antimicrobial
- Antioxidant
- Antidiabetic
- **Multiple Biological Activities**
- Antihypertensive
- Anticancer
- Immunomodulatory

Introduction

Lunasin

Gm2S-1 albumin 2S
43 amino acids

1-22
Unknown function
Posible interaction with deacetylated H4

23-32
Histone H4 binding

33-35
Cell adhesion

36-43
Inhibits H3 acetylation

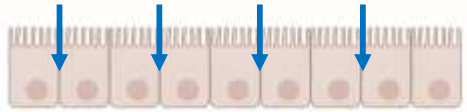
Sources

Soybean and soy products

Barley

Wheat

Absorption →



Pasive paracellular diffusion mechanism

Biological activities → Chemopreventive / Anti-inflammatory / Hypocholesterolemic

Immunomodulatory

Objectives

Main Objective

Evaluation of the antioxidant and immunomodulatory activity of a lunasin enriched soybean extract in murine RAW 264.7 macrophages

Specific Objective 1

Characterization by electrophoresis and Western-Blot of the **protein profile** of the lunasin enriched soybean extract

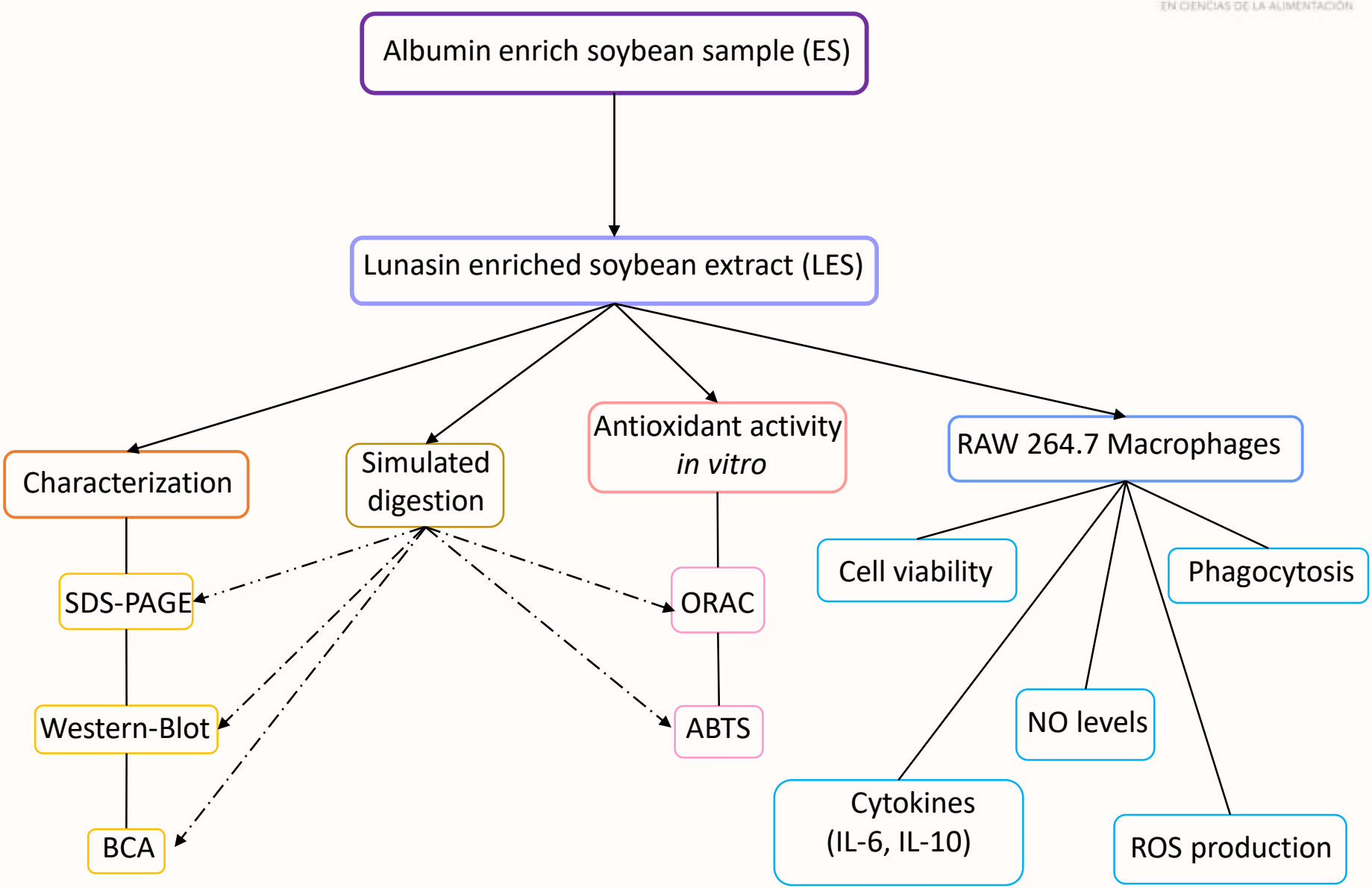
Specific Objective 2

Study of the **behavior** of the lunasin enriched soybean extract under conditions that **simulate the gastrointestinal digestion process**

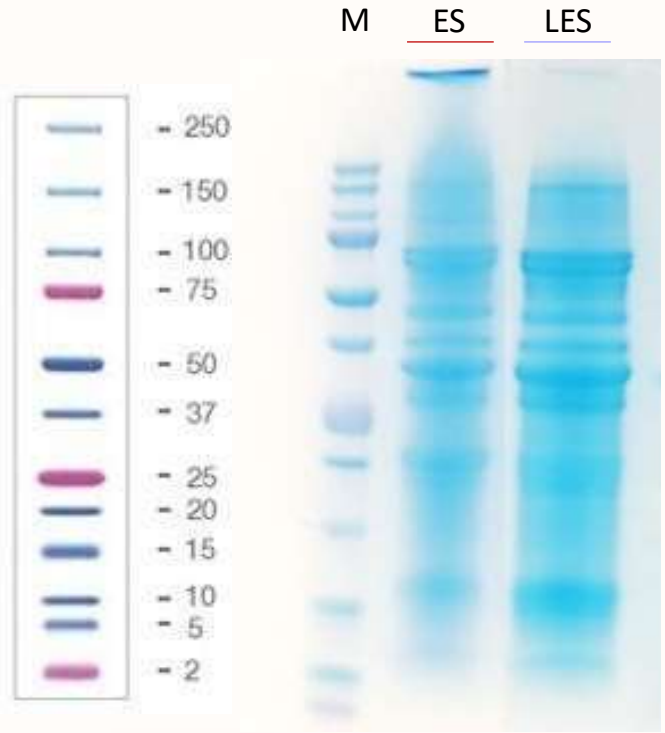
Specific Objective 3

Study of the effect of the lunasin-enriched extract on **biomarkers associated with oxidative stress and the immune response**

Workplan


Albumin enrich soybean sample (ES)
Lunasin enriched soybean extract (LES)
Characterization
Simulated digestion
Antioxidant activity *in vitro*
RAW 264.7 Macrophages
SDS-PAGE
Western-Blot
BCA
ORAC
ABTS
Cell viability
Phagocytosis
NO levels
Cytokines (IL-6, IL-10)
ROS production

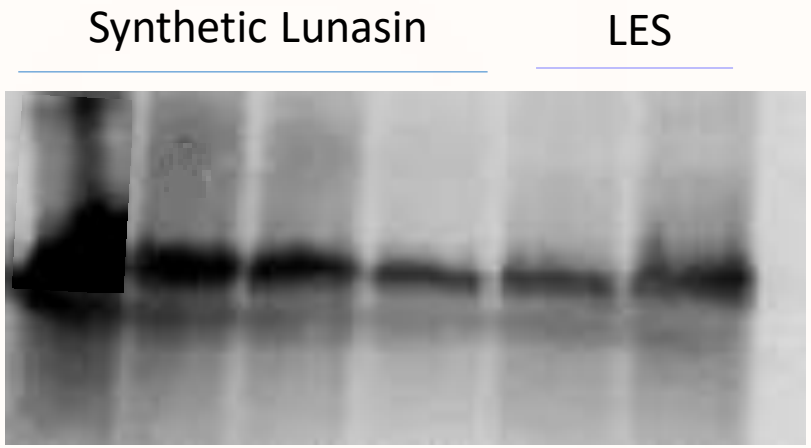
Results: LES characterization



SDS-PAGE electrophoresis

- **Similar profiles** for ES and LES, although bands in LES showed more intensity
- Identification of 4-138 kDa bands

Presence of the major soybean proteins: β-conglycinin, glycinin and their corresponding subunits

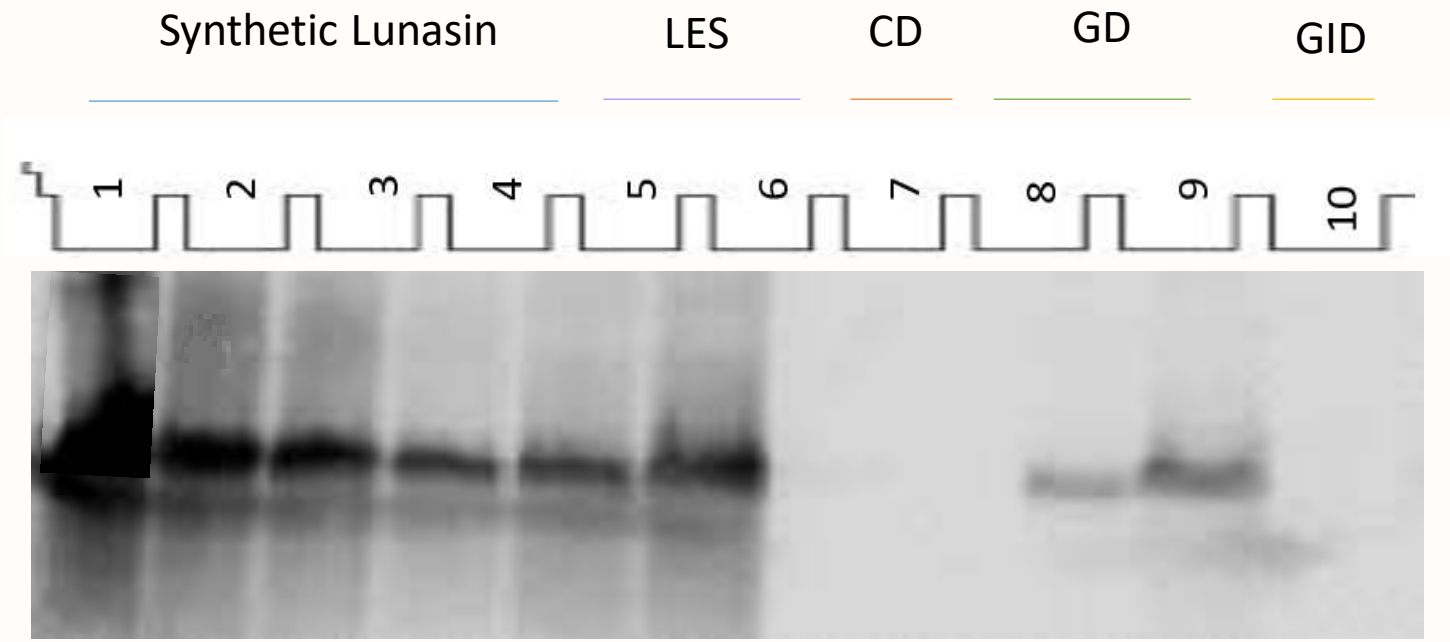


Western-Blot

- Identification of lunasin's monomeric and dimeric forms

16.42 mg lunasin/g of protein
2.07 mg lunasin/g of extract

Results: Behaviour under simulated gastrointestinal digestion



60% of the initial lunasin was detected in **GD**

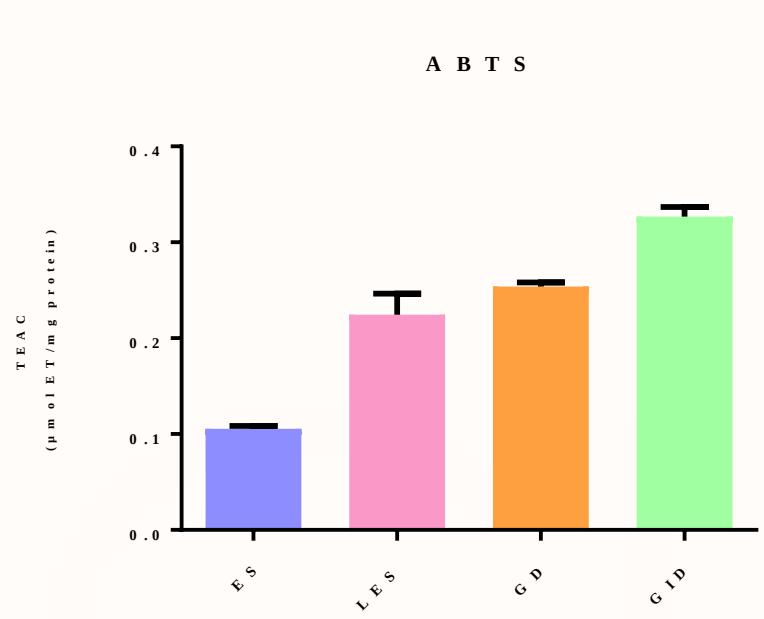
2.88% of the initial lunasin was detected in **GID**

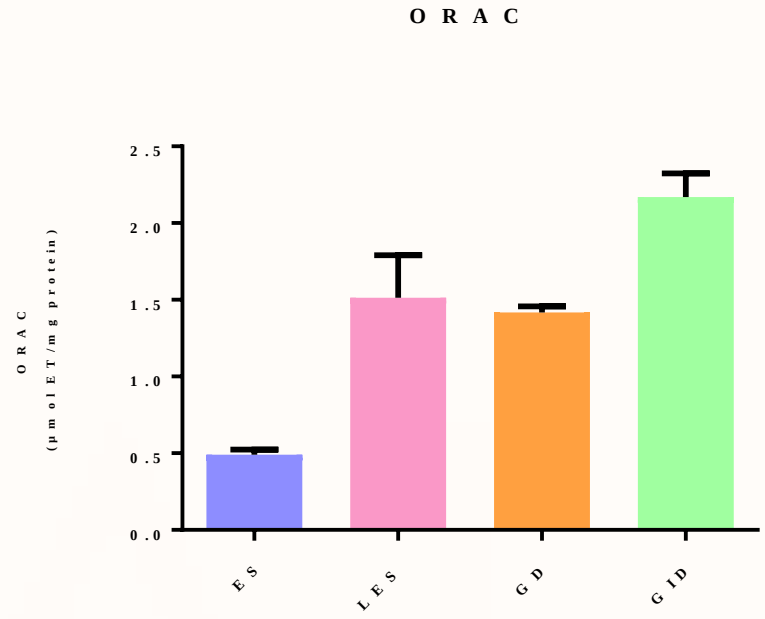
Protease inhibitors (Bowman-Birk inhibitor or Kunitz inhibitor) potentially present in LES **could be responsable for the greater resistance** of lunasin to the digestive process

Results: Antioxidant activity of LES

Neutralizing capacity

ES < LES



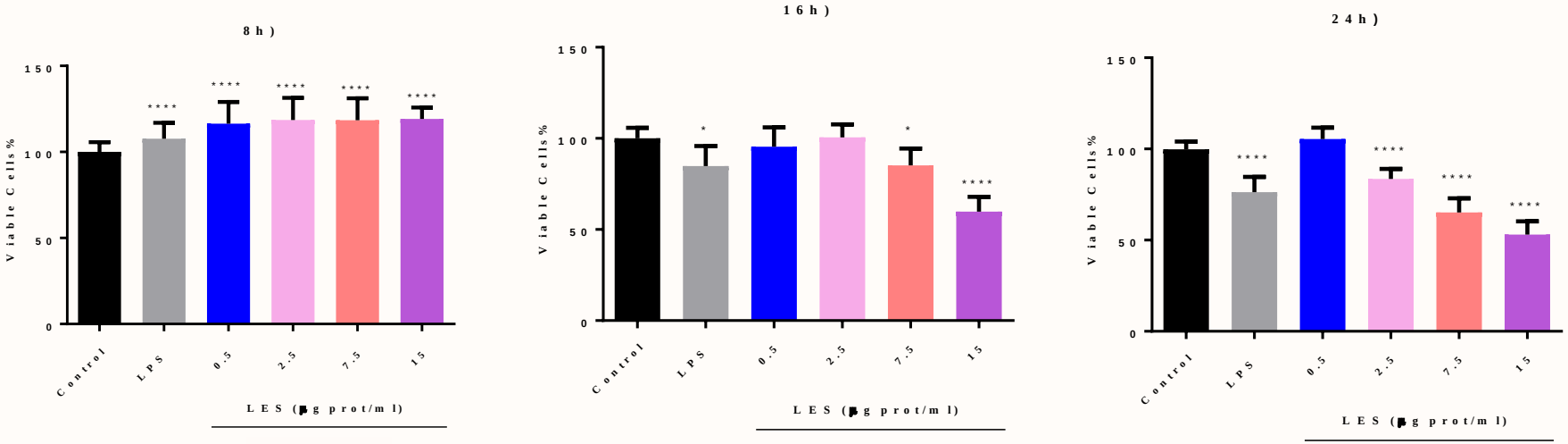


Soluble proteins and small peptides: **responsible for the antioxidant activity**

The antioxidant activity was **higher after the gastrointestinal digestion**

Results: Immunomodulatory activity in macrophages RAW264.7

Cell Viability (MTT assay)

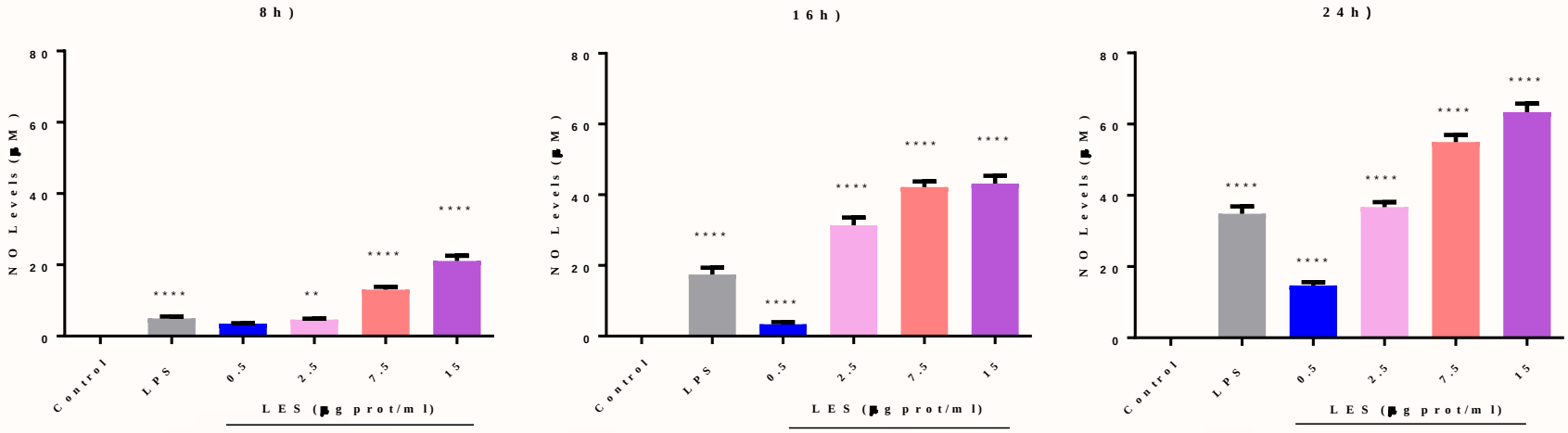


Significant and dose-dependent increase in the % of viable cells was observed after first **8 h**

Longer treatment times resulted in **significant reduction of cell viability**

Results: Immunomodulatory activity in macrophages RAW264.7

NO Production (Griess assay)

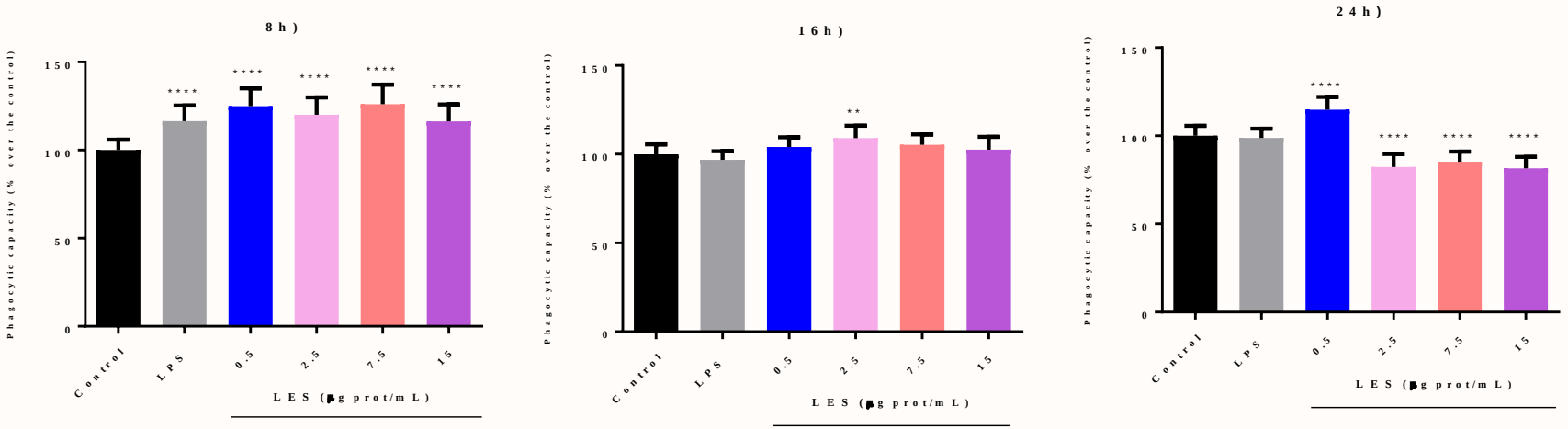


Induction of the **release of NO was provoked** by the treatment with both **LPS and LES**

For **LES**, the **induction** was **dose- and time-dependent**,
reaching values higher than 60 uM at LES doses of 15 ug protein/mL for 24 h

Results: Immunomodulatory activity in macrophages RAW264.7

Phagocytic Activity



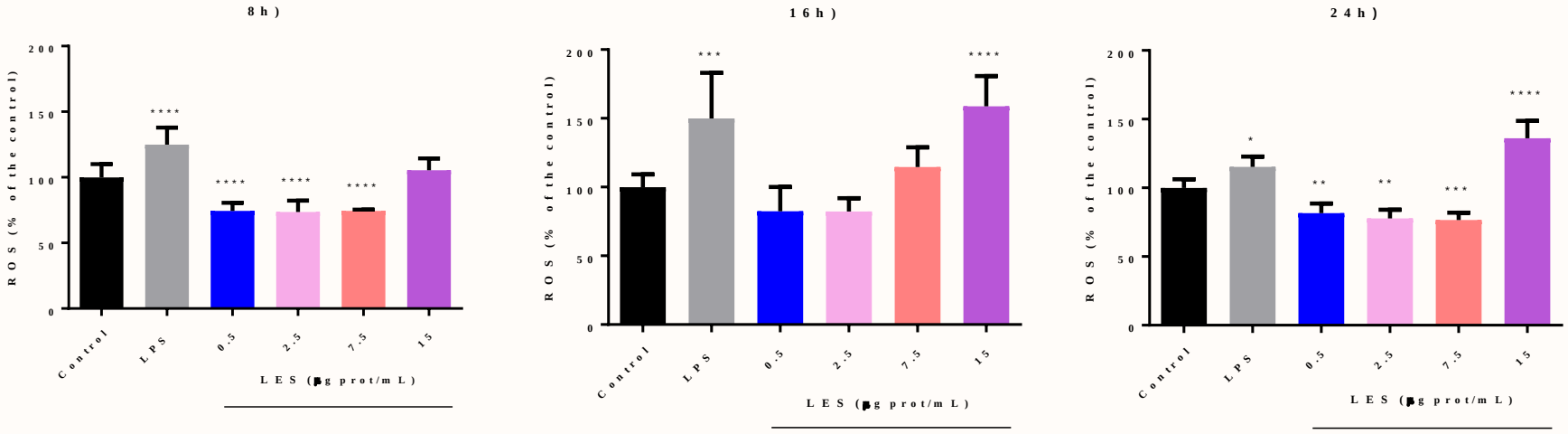
After **8 h** of treatment with LES, the **phagocytic capacity was significantly increased,** with a similar behaviour to that of LPS

After **16 h**, **no changes** were observed **except** for **2.5 µg prot/mL of LES**

At **24h**, only the **lower concentration caused an increase** in phagocytic capacity, **while the higher concentrations** caused a **significant decrease**.

Results: Immunomodulatory activity in macrophages RAW264.7

ROS Production

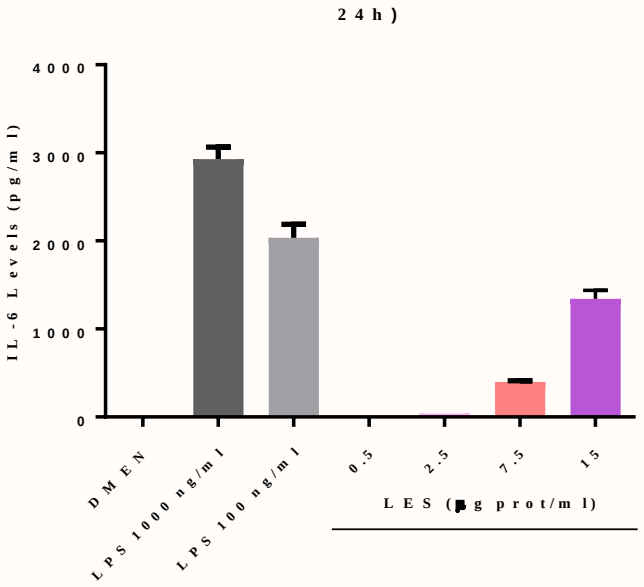


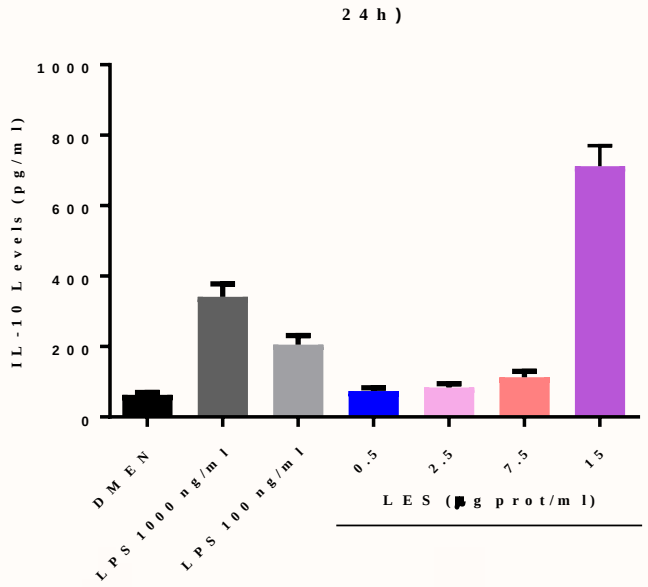
LES at concentrations of **0.5. 2.5 and 7.5 µg protein/mL** exerted a **protective effect against oxidative stress**, decreasing ROS levels at the **three treatment times**

The **highest concentration** caused an **oxidizing effect** at the three times tested, similarly to LPS

Results: Immunomodulatory activity in macrophages RAW264.7

Cytokines Production





LES **induced** the **liberation** of **IL-6** in a **dose-dependent manner.**

LES **induced** the **liberation** of **IL-10** in a **dose-dependent manner.** At LES highest concentration, the levels of IL-10 were even higher than the LPS.

Conclusions

1. An **enrichment** in soluble proteins and small peptides such as lunasin, whose concentration was **2.07 mg lunasin/g of LES,** was achieved. The **protease inhibitors** could exert a **protective effect** against digestive enzymes, allowing lunasin to partially **resist the digestive process.**

2. The **small and medium peptides** were **responsible for the radical neutralizing activity.** After the simulated digestive process, the activity increased, indicating that peptides released during digestive process were more potent as antioxidants.

3. LES presented, in a **dose- and time-dependent manner**, an **immunomodulatory activity** in RAW 264.7 macrophages as demonstrated by changes in the levels of **NO and ROS**, and in the **phagocytic activity** of the cells. LES, also had a **dose-dependent** effect in the liberation of proinflammatory (IL-6) and antiinflammatory **cytokines** (IL-10).