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ANTI-INFLAMMATORY PROFILE OF BIOACTIVE COMPOUNDS DERIVED FROM EXTRA-VIRGIN OLIVE OIL IN ZEBRAFISH MODELS (*Danio rerio*)

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AIM OF THE STUDY

To evaluate the toxicological and therapeutic effects of bioactive compounds present in **extra-virgin olive oil (EVOO)** on pathology **zebrafish** (*Danio rerio*) *in vivo* models. Initially, the toxicological profile of the polyphenols under examination will be assessed to select sublethal concentrations for testing their therapeutic potential on larvae treated, for example, with lipopolysaccharides (LPS) and copper sulfate. The endpoints investigated to evaluate the therapeutic and modulatory effects of the compounds in **inflammatory models** will be primarily molecular (Real-time PCR) and biochemical (spectroscopy methods).

STATE OF THE ART

Extra-virgin olive oil (EVOO) is well-known for its **nutritional qualities and health advantages** [1].

Chemical composition of extra-virgin olive oil (EVOO)	
Monounsaturated fatty acids	55-80 %
Saturated fatty acids	8-14 %
Polyunsaturated fatty acids	4-20 %
Bioactive compounds	1-2 %

220 different substances: hydrocarbons, tocopherols, polyphenols, alcohols, sterols and pigments [2].

Among other EVOO chemicals, the secoiroids **oleocanthal (OLC)** and **oleacein (OLE)** are being studied for their beneficial effects against various diseases such as arthritis [3], neurodegenerative [4] and cardiovascular diseases [5], however their full potential to promote health remains uncertain.

Moreover, the protective effects of OLE and OLC have been studied only *in vitro* [6]. Therefore, further *in vivo* studies are necessary to better understand their therapeutic properties. The zebrafish (*Danio rerio*) is an excellent research model for studying multiple pathologies, including inflammatory [7] and oxidative stress-related diseases [8] as well as for discovering and further investigating the beneficial effects of different molecules to alleviate these conditions. Its optical transparency [9], genetic similarity to humans [10], and the ability to manipulate its genome make this model valuable for biomedical research.

OBJECTIVES AND MILESTONES

A1) Determination of toxicological profile of a green bioactive extra-virgin olive oil extract containing the secoiridoids oleocanthal, oleacein, hydroxytyrosol and tyrosol in the zebrafish model. Acute toxicity tests (FET TESTs) will be conducted on embryonic and larval forms of zebrafish that will be exposed to seven concentrations of the extract, according to OECD guideline n°236, and toxicological endpoints will be calculated (A1.2) (LC50, NOAEL, BMDL-BMDU) using specific software (ToxRat Solutions GmbH, Germany; EFSA web-tool).

A2) Evaluation of anti-inflammatory properties of the extract of extra-virgin olive oil. For this purpose, chemical inflammation models will be created and validated in wild-type and transgenic zebrafish through exposure to LPS and copper sulfate (A2.1). Sub-lethal concentrations of the extract will be tested individually and in combination with the chemicals used to establish the pathology models (A2.2).

A3) Evaluation of anti-inflammatory properties of each bioactive compound present in the extract. To assess the beneficial effects of the compounds under examination (oleocanthal, oleacein, tyrosol and hydroxytyrosol), at least sub-lethal concentrations will be tested in zebrafish larvae treated with pro-inflammatory molecules (A3.1).

A4) Assessment of therapeutic/modulatory effects of the polyphenols in exam. The endpoints investigated to evaluate the therapeutic and modulatory effects of the compounds in different systems will be molecular (Real-time PCR) (A4.1), to evaluate expression of inflammatory gene expression, and biochemical (spectroscopy methods) (A4.2) to assess the activity of different enzymes: glutathione peroxidase, NO synthase, catalase and lipid peroxidation.

A5) Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Activity Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Toxicological profile of extract***																								
	1) FET TESTs																								
	2) Toxicological endpoints																								
A2)	***Evaluation of anti-inflammatory properties of the extract***																								
	1) Creation of inflammatory zebrafish model																								
	2) FET TESTs																								
A3)	***Evaluation of anti-inflammatory properties of single bioactive compound***																								
	1) FET TESTs																								
A4)	***Assessment of therapeutic/modulatory effects of the polyphenols in exam***																								
	1) Enzymatic biochemical assay																								
	2) Molecular assay (PCR)																								
A5)	***Thesis and Paper Preparation***																								

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