TOPICS IN *Caenorhabditis elegans*

Daiana Silva de Ávila
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Daiana Silva de Ávila: Bachelor in Pharmacy (Federal University of Santa Maria-UFSM), Master in Biological Sciences - Toxicological Biochemistry (Federal University of Santa Maria - UFSM), PhD in Biological Sciences - Toxicological Biochemistry (Federal University of Santa Maria), pos-doc in Neurotoxicology at Vanderbilt University. Currently, is associate professor at the Federal University of Pampa (UNIPAMPA) and leads the Laboratory in Biochemistry and Toxicology in *Caenorhabditis elegans*.

Priscila Gubert: Bachelor in Pharmacy Generalist (Federal University of Santa Maria - UFSM), Master in Molecular Medicine (Federal University of Minas Gerais - UFMG), PhD in Toxicology Biochemistry (Federal University of Santa Maria - UFSM; Vanderbilt University, USA; and Albert Einstein College of Medicine, USA). Adjunct Professor at Federal University of Pernambuco and Researcher at Keizo Asami Institute (iLIKA).

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Authors

Aline Castro Silva: Bachelor in Pharmacy (Federal University of Pampa- UNIPAMPA), Master student in Biochemistry (UNIPAMPA).

Amanda Onduras de Andrade: Bachelor in Pharmaceutical Sciences (Federal University of Pernambuco - UFPE).

Ana Maria Vieira da Silva: Undergraduate Pharmacy student (Federal University of Pernambuco- UFPE).

Andy Joel Taipe Huisa: Bachelor in Toxicology (Faculty of Pharmacy and Biochemistry - Universidad Nacional Mayor de San Marcos), Master in Physiological Sciences (Universidade Federal do Rio Grande - FURG), PhD student in Physiological Sciences (Universidade Federal do Rio Grande - FURG).

Douglas Correia de Souza: Bachelor in Biomedicine (President Antonio Carlos University - Unipac), Master in Veterinary Medicine - Biotechnology (Federal University of Viçosa - UFV) and PhD student in Veterinary Medicine - Biotechnology (Federal University of Viçosa - UFV).

Daiana Silva de Ávila: Bachelor in Pharmacy, Federal University of Santa Maria- UFSM), masters in Biological Sciences- Toxicological Biochemistry (UFSM), PhD in Biological Sciences- Toxicological Biochemistry (UFSM), pos-doc in Neurotoxicology at Vanderbilt University. Currently, associate professor at the Federal University of Pampa (UNIPAMPA) and leads the Laboratory in Biochemistry and Toxicology in *Caenorhabditis elegans*.

Daniela Teixeira Rodrigues: Bachelor in Natural Sciences (Federal University of Pampa- UNIPAMPA), Master in Biological Sciences - Toxicological Biochemistry (Federal University of Santa Maria - UFSM), and PhD student in Biological Sciences - Toxicological Biochemistry (Federal University of Santa Maria - UFSM).

Doralice Conceição da Paz Neta: Undergraduate Biomedicine student (Federal University of Pernambuco- UFPE).

Elias Almeida Silva Barbosa: Undergraduate Biomedicine student (Federal University of Pernambuco). Intern at the Harvard Stem Cell Institute. Research Assistant and trainee at the Dana Farber Cancer Institute - Cancer Immunology and Virology Department. Research Assistant at iLIKA and FIOCRUZ-PE.

Fernando Silveira Rocha: Undergraduate Biology student (Federal University of Pernambuco - UFPE).

Flávia Suelen de Oliveira Pereira: Bachelor in Biomedicine (CNEC College Center- Santo Ângelo), Master in Biochemistry (Federal University of Pampa- UNIPAMPA) and PhD student in Biochemistry (Federal University of Pampa- UNIPAMPA).

Francine Côa: Bachelor in Environmental Technology (State University of Campinas - Unicamp), Master in Aquaculture and Fisheries (Fisheries Institute - IP), specialist in Project Managment and PhD student in Chemistry (University of São Paulo - USP).

Gabriel Pedroso Viçozzi: Bachelor in Pharmacy (Federal University of Pampa-UNIPAMPA), Master in Pharmaceutical Sciences (Federal University of Pampa - UNIPAMPA) and PhD student in Biological Sciences - Toxicological Biochemistry (Federal University of Santa Maria - UFSM).

Gustavo Roberto Villas-Boas: Bachelor in Pharmacy (Catholic University Center of Southwest Paraná - UNICS), Master in Pharmacology (Federal University of Paraná - UFPR), PhD in Health Sciences (Pharmacology) (Federal University of Grande Dourados - UFGD). Adjunct Professor at Federal University of Western Bahia and lead researcher at the Research Group on Development of Pharmaceutical Products (P & DProFar).

Heloísa Aiolfi Padilha: Bachelor in Pharmacy (Federal University of Pampa-UNIPAMPA) and Master student in Biochemistry (Federal University of Pampa-UNIPAMPA).

Isabel Cristina Oliveira Fernandes: Bachelor in Pharmacy (Mauricio de Nassau University Center - Uninassau) and Master student in Biology applied to Health (Federal University of Pernambuco - UFPE)

Iverson Conrado Bezerra: Undergraduate Biomedicine student (Federal University of Pernambuco - UFPE). Scientific Initiation at Keizo Asami Institute (iLIKA).

Laura Vitória dos Santos: Undergraduate Biology student (Federal University of Pernambuco - UFPE). Scientific Initiation at Keizo Asami Institute (iLIKA).

Lucas Aleixo Leal Pedroza: Undergraduate Biomedicine student (Federal University of Pernambuco - UFPE). Intern at Instituto Nacional do Câncer - INCA.

Maria Clara Cavalcante Gomes: Undergraduate Biomedicine student (Federal University of Pernambuco - UFPE).

Maria Eduarda Oliveira de Souza: Bachelor in Pharmacy (Federal University of Pampa-UNIPAMPA), Master student in Biochemistry (Federal University of Pampa - UNIPAMPA).

Marina Meirelles Paes: Bachelor in Pharmacy - Generalist (Faculty of Medicine of Campos-FMC), Master in Vegetable Production (Universidade Estadual do Norte Fluminense - UENF), PhD in Natural Sciences (Universidade Estadual do Norte Fluminense - UENF). Adjunct Professor CI at the Federal University of Western Bahia (UFOB).

Michael Aschner: Bachelor in Natural Sciences (University of Rochester), Master in Anatomy (University of Rochester), PhD in Neurobiology and Anatomy (University of Rochester), pos-doc in Environmental Health Sciences Center, and Professor of Molecular Pharmacology, Neuroscience, Pediatrics (Albert Einstein College of Medicine of Yeshiva University).

Matheus Chimelo Bianchini: Bachelor in Pharmacy (Federal University of Pampa- UNIPAMPA), Master in Biochemistry (UNIPAMPA), PhD student in Biochemistry (UNIPAMPA) and substitute professor at UNIPAMPA.

Natália Silva Jardim: Bachelor in Pharmacy (Federal University of Santa Maria- UFSM), Master in Biological Sciences- Toxicological Biochemistry (UFSM), PhD in Biological Sciences- Toxicological Biochemistry (Federal University of Santa Maria - UFSM) and pos-doc at Federal University of Pampa (UNIPAMPA).

Priscila Gubert: Bachelor in Pharmacy Generalist (Federal University of Santa Maria - UFSM), Master in Molecular Medicine (Federal University of Minas Gerais - UFMG), PhD in Toxicology Biochemistry (Federal University of Santa Maria - UFSM; Vanderbilt University, USA; and Albert Einstein College of Medicine, USA). Adjunct Professor at Federal University of Pernambuco and Researcher at Keizo Asami Institute (iLIKA).

Rosana Pereira Nobre de Lima: Undergraduate Biomedicine student (Federal University of Pernambuco- UFPE).

Tábata Martins de Lima: Bachelor in Biology (Federal University of Rio Grande - FURG), Master in Physiological Science (Federal University of Rio Grande - FURG), PhD in Physiological Science (Federal University of Rio Grande - FURG), and Professor at State University of Minas Gerais (UEMG).

Victória Regina da Silva: Undergraduate Biomedicine student (Federal University of Pernambuco - UFPE).

Preface

Caenorhabiditis elegans is a free living non-parasitic nematode that was brought to the spotlight by Sydney Brenner in 1965. Initially, it was used to unravel developmental aspects of animals, but then the simplicity and easy maintenance of the worm made it an excellent tool for genetic interventions. Since then, it has been possible to sequence its whole genome, characterize the nervous system's wiring, observe *in vivo* the expression of proteins by tagging them with a green fluorescent protein (GFP) and generate knockout strains to study the role of specific genes. Furthermore, it is possible to humanize worms by inserting human genes and making them express mutant beta-amyloid, for example, making this worm a model to study Alzheimer's Disease. Notably, recent studies have established that approximately 70% of the human genome is conserved in *C. elegans* and several pathways are highly similar, such as cell fate, cell division, innate immunity, neurotransmitters synthesis and degradation, insulin-like pathway, apoptosis, necrosis, energy production, just to name a few. In addition, these features make it a great model for predicting biological effects from the simpler species to the most complex, which is useful for toxicological assessments and drug screenings. Considering its ubiquitous presence in the environment, it is also a great model for ecotoxicological assessments.

The Topics in *C. elegans* is a production of the Academic League of studies in *Caenorhabditis elegans* - LAECe (from Portuguese, *Liga Acadêmica de Estudos em Caenorhabditis elegans*), Federal University of Pernambuco - UFPE. LAECe is formed by undergraduate and graduate students who have decided to study this amazing experimental model together. As part of an extension program, LAECe focuses on scientific disclosure for the neighborhood community by taking *C. elegans* to schools and science fairs, organizing a *C. elegans* symposium (SIMPECe, Simpósio Pernambucano de *C. elegans*), and providing interesting lectures online (LIKA-UFPE Institute Youtube Channel).

Topics in *C. elegans* was a big challenge for all of us. For many students, it was their first experience in writing a book chapter. There was no limit on the chaptersubjects which were entirely chosen by the students. That is why the reading will become very rich and full of important aspects of *C. elegans* research. We are sure youare going to enjoy the nice work from these brilliant young scientists.

Welcome to the LAECe community!

Daiana Silva de Ávila & Priscila Gubert

Summary

Chapter 1

The neurotoxic potential of environmental pollutants in the *Caenorhabditis elegans* model

Iverson Conrado Bezerra
Doralice Conceição da Paz Neta
Fernando Silveira Rocha
Gabriel Pedroso Viçozzi
Isabel Cristina Oliveira Fernandes
Laura Vitória dos Santos
Maria Clara Cavalcante Gomes
Natália Silva Jardim

Abstract:

Neuronal damage is a concerning condition since the nervous system controls several functions in several species. Besides the genetic factor involved in some neurodegenerative processes, environmental pollutants have been identified as causing neurotoxic effects. Considering the increasing discharge of these toxicants, the studies on their mechanisms are essential to implement policies to control their release and find antidotes. *C. elegans* has been a great tool in this endeavor because of the availability of mutant strains, some of which allow the observation of neuronal viability *in vivo*. In addition, *C. elegans* belongs to the soil biota and therefore can be used to assess ecotoxicology. Because of its homology to mammals, the studies can be used to predict effects in other species. This chapter reviews worm neurobiology, the methods used to assess neurotoxicity, and we focus on the results found following exposure to carbamates, organophosphates, Tetrabromobisphenol A (TBBPA), microplastics, graphene oxide, and metals.

keywords: neurotoxicity; *C. elegans*; TBBPA; oxide graphene; microplastics, metals

1. Introduction

As a result of the rapid process of industrialization, the severity of environmental pollution has been aggravated. Among the several pollutants, metals, pesticides, and microplastics are reported as the most present in the environment, primarily due to their persistence and ability to bioaccumulate. Thus, it is suggested that these exert negative impacts on the ecosystem and on humans since it is also reported that toxicity can be transferred by subsequent generations to contamination

(CHEN *et al.*, 2019).

In agricultural production, the application of pesticides is widely used for pest control, including carbamates and organophosphates. Although the effective action and preventive function against pests in the crops, the lack of specificity targets can cause severe and lasting effects on animal species, humans, as well as the environment. Countries such as Brazil, the United States of America, and China are the largest agricultural producers and exporters globally, also representing the largest consumers of pesticides, with estimated amounts ranging between 827 million and 3.9 billion kilograms (DONLEY, 2019; WANG *et al.*, 2021).

Despite the absence of acute toxicity, plastics have become a major environmental burden. It is estimated that approximately eight million tons of plastics per year are discarded and end up in the oceans and about 5.25 trillion plastic particles circulate in their surface waters. With the plastic degradation processes, sunlight and wave action microplastics are generated, which are considered emerging contaminants, representing plastic fragments with a variable size from 1 to 5000 µm (FICOCIELLO *et al.*, 2021). Studies indicate that due to the ease of ingestion by various organisms due to their small size, they can accumulate in humans through the food chain (YU *et al.*, 2020; KIM *et al.*, 2020). The chronic toxic effects are diverse, and these are related to the possibility of crossing cell membranes and causing damage to animal tissues, neurotoxicity, in addition to evidence that they can interfere with the plant photosynthesis and microalgae growth (Hu *et al.*, 2020; CHEN *et al.*, 2020; CHEN *et al.*, 2021).

Among the ranking World Organization of Health (WHO) of the 10 chemicals of public health concern, heavy metals stand out. Some light metals are for example zinc, copper elements, and some essential items that serve as cofactors for enzymatic reactions. Other metals, including lead and cadmium, do not occur naturally in biological systems. Their mechanisms of toxicity are diverse and may include competition for endogenous enzyme cofactors, effects on ion channels, or oxidative damage. As these easily cross the blood-brain barrier, they can also cause neurotoxicity (ZHOU *et al.*, 2017; BARAGAÑO *et al.*, 2020). One of the consequences related to rapid industrial development is the increase in the global consumption of tetrabromobisphenol

A (TBBPA), one of the most common brominated flame retardants, widely available and frequently used in industrial products. A large amount of TBBPA is released into the environment within the manufacturing and useprocesses, therefore, TBBPA has often been detected in various environmental matrices. Toxicological research demonstrates that TBBPA has a high risk of endocrine, reproductive, neurobehavioral and developmental toxicity in organisms (TIAN *et al.*,2015, LUI *et al.*, 2020).

The promising uses of graphene oxide (GO) raise concerns about its risks to human health and the environment. The environmental presence of this material may result from its industrial use or the improper disposal of waste or its products. GO is an extremely oxidative form of graphene acquired by the chemical exfoliation of graphite. It is a valuable material for graphene-based applications in electronics, optics, chemistry, energy storage, and biology. The biomedical application and toxicological effects of GO are still not well defined (DIDEIKIN *et al.*, 2019, CLEMENTE *et al.*, 2017). Thus, taking into account the risks to human health, as well as to the environment, it is extremely important to investigate possible chronictoxicities and the mechanisms associated with exposure to environmental contaminants (HUNT, 2017; LIU *et al.*, 2020).

In this sense, the nematode *Caenorhabditis elegans* (*C. elegans*) has been used as a widely explored animal model for investigating the molecular and cellular aspects of various human diseases (QUEIRÓS *et al.*, 2019). Recently, *C. elegans* was used as a model to verify the *in vivo* toxicity of carbamates, bisphenol A, microplastics, and graphene oxide, among other environmental contaminants, which is possible due to its high homology with humans, short life span, low cost for maintenance, transparent body and sensitivity to pollutants (HU *et al.*, 2021; QUIJANO *et al.*, 2022). Toxic effects that occur in humans can be verified in *C. elegans*, such as lethality, reduction in reproduction rates, delayed development, increased oxidative stress, and cellular apoptosis (KIM *et al.*, 2019) (ZHOU *et al.*, 2016). Of note, neurotoxicity is one of the main effects caused by these toxicants, therefore impairing many essential functions of the worm life. Therefore, in the present review, we aim to describe the studies that have evaluated the neurotoxicity caused by these environmental contaminants using

the animal model *C. elegans*, making it possible to better understand their potential risks to human and environmental health.

2. Neurobiology of *C. elegans*

C. elegans is a non-pathogenic nematode just over 1 mm in length that feeds on soil fungi, bacteria, and decaying fruit.. It has a fast life cycle (3 days at 25°C from egg to adult), increasing in size along four larval stages (L1, L2, L3, and L4), with two sexes (male and hermaphrodite) being distinguished in the last larval stage. In addition, under starvation, it can go to a stress-resistant larval stage called Dauer, which slows its growth rate but promotes greater viability in the presence of toxins. Wild-type *C. elegans* has two sexual forms: self-fertilizing hermaphrodites and males. Hermaphrodites can produce up to 300 of their progenies, which are fertilized by stored sperm. If mated with males, hermaphrodites can produce ~1000 offspring, indicating that sperm produced by hermaphrodites are a limiting factor in self-fertilization (CORSI *et al*., 2015).

C. elegans toxicity assays provide expressive data from an entire animal with intact and metabolically active digestive, reproductive, endocrine, sensory, and neuromuscular systems (HUNT., 2016). Furthermore, this model shows significant cellular and molecular processes similarities with other animals over evolutionary time. 60-80% of human genes have an ortholog in the *C. elegans* genome (KALETTA AND HENGARTNER., 2006), and 40% of genes known to be associated with human diseases have clear orthologs in the *C. elegans* genome (CULETTO AND SATTELLE., 2000) and at least 38% of the genes encoding the *C. elegans* protein predicted orthologs in the human genome (SHAYE AND GREENWALD., 2011).

Because of its nervous system, *C. elegans* has become an important model for studying neurobiological issues. Of all the tissues in *C. elegans*, the nervous system is one of the most complex, comprising around 40% of its total somatic cells. The hermaphrodite worm has 302 neurons, the adult male has 383, and there are 56 glial cells. The neuronal organization is based on ganglia in the head, ventral cord, and tail regions, structurally similar to the spinal cord of mammals. The whole nervous system has been mapped, and the connectome has been described (BRADLY., 2018). All of the

neurons are well described anatomically, thus assessing the impact of exposure to certain compounds on signaling, morphological and genetic aspects (Figure 1). The understanding of the development of the nervous system in *C. elegans* is still well studied, as it is not yet clear whether there is a specific lineage for each type of neuron since when a known cell is compromised within the system, it can be recovered by a process known as "transdifferentiation," in which a non-neuronal cell can differentiate into a neuronal one (HOBERT., 2010).

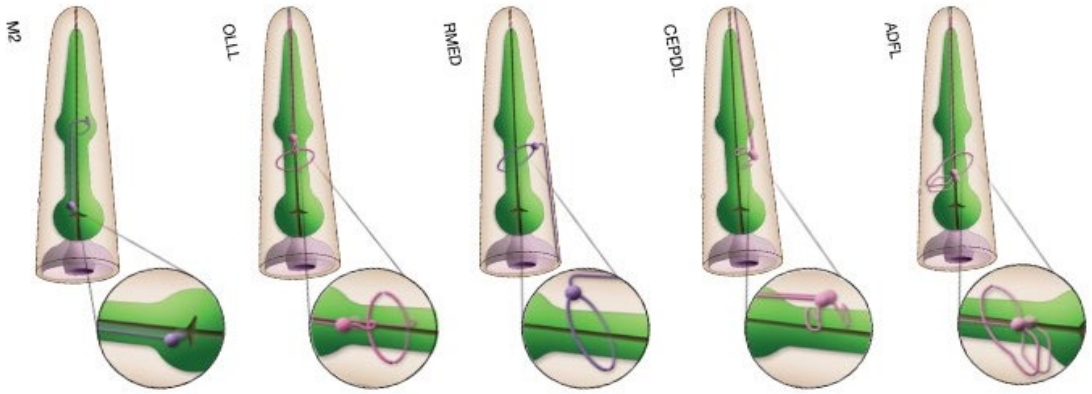

Figure 1. Morphological and structural aspects of some neurons in *C. elegans* (WormAtlas adapted). M2 neurons are located in the posterior pharyngeal medulla, producing and releasing acetylcholine. Mechanosensory neuron, the OLLL is located in the head and secretes the neuropeptide glutamate. ADFL are sensory and serotonergic neurons. RMED and CEPDL are located in the head, releasing GABA and Dopamine, respectively.

Its neurons present great complexity in information processing, in which a neuron can have within its structure up to 14 different receptors with different forms of functioning, which may include ionotropic and metabotropic signaling (DUGGAN AND CHALFIE., 1995). The different types of neurons and these neurotransmitter systems (acetylcholine (ACh), glutamate (Glu), γ-amino butyric acid (GABA), dopamine (DA) and serotonin (5-HT)) have determined functions that are crucial for their development, feeding, reproduction, and locomotion, for instance (Table 1) (HOBERT., 2013). The formation, trafficking, and release of neurotransmitters from the synaptic vesicles and their clearance from the synaptic cleft are highly conserved processes in relation to mammals. Genetic studies have demonstrated that several signaling pathways have

been discovered to be responsible for distinct behaviors in *C.elegans* (RANKIN., 2002; XU *et al*., 2017). Hence, if any change in the worm's behavioral pattern is observed, it can be predicted that possible damage is occurring in specific (s) neuron (s).

Table 1. Neurotransmitters and their roles in the behavioral control of *C. elegans*.

Neurotransmitters	Biological functions
Acetylcholine (ACh)	An essential neurotransmitter, controlling excitation of body-wall muscle, pharyngeal pumping and required for locomotion and feeding (TREININ; JIN, 2021).
Glutamate (Glu)	Glutamate is an important excitatory neurotransmitter. It mediates the processes of locomotion, spontaneous reversal and regulation of oxidative stress in neurons (YU; CHANG, 2022).
γ-amino butyric acid (GABA)	GABA has an inhibitory role in the nervous system, promoting the inhibition of contraction of the ventral and dorsal body wall muscle during locomotion. Stimulate the enteric muscle during defecation. (SUTHAKARAN *et al*., 2021).
Dopamine (DA)	DA signaling modulates the locomotor behavior of worms, and also allows animals to search efficiently for new food sources (CHOU *et al*., 2022).
Serotonin (5-HT)	Serotonin-mediated signaling modifies the response of *C. elegans* regarding its locomotion profile and egg-laying control (KOPCHOK *et al*., 2021).

3. Methods to evaluate neurotoxicity in *C. elegans*

Assessing damages to the neuronal system of different species is a significant challenge when substances deposited in the environment are studied. Usually, researchers mimic these environments in the lab to verify their impacts on the worms. In this context, *C. elegans* has been used to evaluate the neuronal toxicity of different compounds such as pesticides, TBBPA, microplastics (MPs), GO, and metals (QUEIRÓS *et al*., 2021; LIU *et al*., 2020; CHEN *et al*., 2021; KIM *et al*., 2020), which are known neurotoxic substances. A very elegant approach is to use transgenic strains that have been constructed and are available in the *Caenorhabditis* Genetics Center (CGC) or by donation directly from the laboratory source. Table 2 demonstrates someexamples of

these strains and for which purpose they are designed.

Table 2. Main transgenic strains used to evaluate neuronal morphology in *C. elegans*.

Strain	Genotype	Phenotype
BY200	vtIs1(*dat-1*p::GFP; *rol-6*)	Dopaminergic neurons marked with GFP.
LX929	vsIs48 [*unc-17*::GFP]	Cholinergic neurons marked with GFP.
GR1333	yzIs71 [*tph-1*p::GFP + *rol-6*(su1006)]	Serotonergic neurons marked with GFP.
EG1285	oxIs12 [*unc-47*p::GFP + *lin-15*(+)]	GABAergic neurons marked with GFP.
OH441	otIs45 [*unc-119*::GFP]	Pan-neuronal neurons marked with GFP.

The strains have a fluorescent protein that can be green (GFP), yellow (YFP), or red (mCherry), tagging different types of neurons or a group of neurons. Then, to evaluate the integrity of these neurons, it is required first to expose the nematodes to the toxicant in a suitable exposure protocol, taking into consideration parameters such as larval stage, time, and type of exposure. Once the protocol is established, the following step is preparing the worms in microscope slides to take images in a fluorescent microscope. Finally, depending on the type of neuron, it is possible to evaluate some specific parameters. For example, it is possible to quantify the fluorescence intensity present in the neurons and evaluate their integrity. It is alsopossible to observe different alterations in the neuronal morphologies. For instance, in strain LX929, it is possible to classify axonal alterations such as outgrowth, branching, Y-shaped neurons, and others (SOARES *et al.*, 2020).

Behavior assays are an indirect way to assess neurotoxicity because they are related to neuronal systems. In *C. elegans,* the behavioral assays are relatable to the ones performed in rodents. Locomotion assays can be used, such as trashing (in solid medium) and swimming (in liquid medium), to evaluate cholinergic and GABAergic function (KALINNIKOVA *et al.*, 2016; IKEDA *et al.*, 2020). It consists of evaluating the worms' speed, traveled distance, movement angle, and other parameters following exposure to a toxicant. Several available software allows quantifying these parameters

(IBÁÑEZ-VENTOSO *et al.*, 2016; NUSSBAUM-KRAMMER *et al.*, 2015). Furthermore, an exciting assay to evaluate cholinergic response is the egg-laying induced by levamisole, a cholinergic agonist that, when bound to its receptor, induces the hyper contraction of the vulval muscle and the release of the eggs from the uterus (WANG *et al.*, 2018). If the worms do not respond to levamisole, this can indicate cholinergic damage.

In addition, the worms have chemosensory neurons (glutamatergic and serotoninergic) that evaluate the sensorial response and memory acquisition. The basics of the assay for sensorial response consists of adding an attractive odorant tothe plate and a repellent and evaluating if the worm moves towards the attractive orto the repellent. If there is damage, the nematode goes to the repellent independently if it is present due to damage to the chemosensory neurons (BARGMANN., 2006). To assess short and long-term memory, the worms have to be previously exposed to an attractive and then transferred to another plate with a neutral molecule and the attractive. If the worm moves towards the attractant, the treatment's memory is regular or improved (QUEIRÓS *et al.*, 2021). It is also possible to evaluate thermotolerance and touch response, which also evaluate glutamatergic sensory neurons.

Assays such as slow basal, swimming-induced paralysis, defecation, and pharyngeal pumping are used to evaluate the dopaminergic system. Many of these assays are related to Parkinson's (PD) and Alzheimer's (AD) disease models (COOPER AND VAN RAAMSDONK., 2018; WANG *et al.*, 2018). The slow basal assay is based on the premise that when worms find food, the neurons release dopamine, reducing body bends to feed. The movements of the nematode are counted in the NGM medium, and the number of bends in the presence and the absence of food are calculated and compared to the unexposed worms. The same principle is applied to the swimming-induced paralysis assay. However, in this case, the movements are counted in a liquid medium (M9 buffer, for example). Dopamine can be added to the medium to induce paralysis and evaluate the dopaminergic integrity. The time between defecation movements can also be scored since worms have a strictly regulated bowel movement, with constant cycles that vary from 40-42s. The numberof pharyngeal pumpings can be scored in a determined period. It may also indicate neuronal

damage, although unspecific, since several neurons can control this function in association with dopamine. These assays can be recorded to allow the researcher to observe the behaviors of many animals simultaneously. On the contrary, if the worms are not recorded, it is recommended to evaluate one animal per time and per assay to minimize the errors and mistakes that can be made.

4. Neurotoxicants in *C. elegans*

4.1 Carbamates and organophosphates

Pesticides are a large group of heterogeneous chemical products that increase food production and decrease foodborne diseases and pathogens. However, depending on the agent and time of exposure, they can pose a risk to human and animal health. Pesticides are found in food, water, air, houses, workplaces, gardens, and lawns (WEISS *et al*., 2004).

Extensive use of pesticides creates toxicological and environmental impacts on non-target organisms, which can severely affect cholinergic neurons in higher animals and contribute to the development of PA, DA, and male sterility in humans (GOVINDARAJAN *et al*., 2019). Furthermore, it affects other non-target animals such as *C. elegans*, which in the environment live in soil and water reserves close to plantations as a free nematode. Studies using organophosphate pesticides and evaluating the nematodes' damage are widely used. In a study that evaluated the effects of quinalphos (QP), an organophosphate pesticide widely used in agricultural practices, genes associated with locomotion (*unc-47* and *unc-13*) and pharyngeal pumping (*egl-30*) were up-regulated, whereas genes related to epigenetic modulation (*utx-1*), and oxidative stress (*daf-2, daf-16, age-1,* and *glod-4*) were significantly down-regulated in *C. elegans*. The data indicate that oxidative stress plays a central role in QP- induced neurotoxicity (GOVINDARAJAN *et al*., 2019).

The dopaminergic effects of the organophosphorus insecticide monocrotophos (MCP) were evaluated along with glucose supplementation, which could lead to an accentuation of the damages. The worms were acutely exposed to MCP with an addition of 2% of glucose (111 mM) in the NGM medium, two life-periods considered, 8 and 13 days old. Authors observed that older worms (13 days old) were more

susceptible to the pesticide by presenting decreased chemotaxis index, impaired memory, increased ROS levels and carbonyls, and decreased Adenosine triphosphate (ATP) levels. Also, mutant strains related to the dopaminergic system were used, such as NL5901 (pkIs2386) and BZ555 (*egls1-dat-1p*::GFP), and the pesticides caused damageto these neurons in a concentration-dependent manner. The authors also observed that the high intake of glucose aggravated the effects of MCP and the dopaminergic damage (SALIM *et al.*, 2018).

The neurotoxicity and developmental effects of the widely applied insecticide methomyl were investigated by a multi-level approach (behavior and biometry, biochemical alterations, and neurodegeneration) in *C. elegans* upon a short-term exposure (1 h) and a post-exposure period (48 h). The type of movement was significantly altered in methomyl-exposed worms and biometric parameters (worms frequently idle and moving more backward than controls; small body area, length, and wavelength) (QUEIRÓS *et al.*, 2021).

In a study evaluating the combined toxicity of carbamate (CMs) mixtures as well as organophosphorus (OPs) mixtures using locomotion behaviors as endpoints, authors measured the body bend, head thrash and swimming speed inhibition of the nematodes exposed to carbofuran (CAR), methomyl (MET), chlorpyrifos (CPF), and triazophos (TAP) and their binary mixtures. The worms were exposed for 48 h to the four pesticides and six binary mixtures (CAR-MET-R1,2,3) (CPF-TAP-R1,2,3). The results suggest that the organophosphorus interfered in the evaluated endpoints more significantly than the carbamates, and the OPs mixtures had a low-dose addictive action and a high-dose antagonism. The CMs mix presented a synergic effect at low doses. In addition, statistical analysis indicated that TAP is the principal contributor to the toxicity of the mixtures (WANG *et al.*, 2021).

Chlorpyrifos (CPF) is widely used in agriculture, and its neurotoxicity is well established. Several studies using different species demonstrated its neurotoxic effects, mainly due to the cholinergic effect. In this sense, a study exposing L4 worms for 24 or 72h demonstrated reduced locomotion and impaired reproduction, two functions regulated by the cholinergic system (RUAN., 2009). Another study using young adults

exposed to CPF for 24h demonstrated inhibition of acetylcholinesterase activity, increased expression of *cep-1* (a *p53*-like protein), and reduced antioxidant-related genes (ROH., 2008). Even at non-cholinergic concentrations, CPF causes neurobehavioral deficits in exposures in different life phases (SILVA MH., 2020).

Moreover, the effects of photolysis and the modifications that this process may cause in the pesticide have been evaluated in a study performed by (CAO *et al*., 2020). The authors evaluated the degradation of CPF at 20 and 50 μg L^{-1}, and posteriorly the integrity of GABAergic neurons using the mutant strain EG1285 (juIs76[*unc-27*p::GFP + *lin-15*(+)] II) and cholinergic neurons using the mutant strain LX929 (vsIs48 [unc- 17::GFP]) were evaluated. Also, locomotion endpoints such as head thrash, body bend, and pumping rate were assessed. At the end of the experiments, the authors proposed two degradation routes of CPF leading to thirteen products. After 1 h exposure, there were more accentuated neurotoxic effects in the evaluated neurons compared to worms exposed to the unirradiated solutions. The concentration of 50 μg L^{-1} caused more damage to the animals' locomotion than the unirradiated solutions at 20 μg L^{-1} (CAO *et al*., 2020).

4.2 Tetrabromobisphenol A (TBBPA)

Tetrabromobisphenol A (TBBPA) is currently used as a brominated flame retardant, often in the composition of plastics, textiles and electronic materials (ZHOU; YIN; FAIOLA, 2020). The diffusion of TBBPA occurs in the most different biotic and abiotic systems and matrices, being found in air, water, soil, among others (HUANG *et a*l., 2014). Bioaccumulation occurred in animals exposed to TBBPA, mainly due totheir intake promoting accumulation and damage to the food chain, being this process facilitated by the octanol/water partition coefficient (log Kow = 4.5-6.5) and long half- life (average of 2 months) in water, soils and surfaces (SHI et al., 2017; LI *et al.*, 2015). There is a great global demand for the use and production of TBBP, exceeding 100,000 tons in the global market according to the international agency for cancer research(IARC WORKING GROUP ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS, 2018).

The neurotoxic, cytotoxic, and immunotoxic potential threatens the health of different species since structurally TBBPA has similarities to thyroxine and steroidsand is considered a disruptor of the endocrine system (HOU et al., 2019; ZHU et al., 2020).

TBBPA was found in human breast milk (37.34 ng/g) and also in umbilical cord serum (649.45 ng/g) (CARIOU et al., 2008). Potentially harmful to the nervous system, low-concentration TBBPA caused the release of lactate dehydrogenase (LDH), stimulates caspase-3 activity, and induced DNA fragmentation in primary rat cerebellar granule and in cortical neurons in vitro (WOJTOWICZ; SZYCHOWSKI; KAJTA, 2014; ZIEMIŃSKA *et al.*, 2012).

In ecotoxicological evaluations of TBBPA exposure, *C. elegans* presented different behavioral changes in locomotor balance, neuromodulation status and increase of reactive species. The transgenerational effects of exposure of animals to TBBPA caused in G1 worms a significant decrease in growth compared to control ($p < 0.01$) with increasing concentrations from 100 to 1000 µg L^{-1}. Changes in brood size were also observed, animals in G1 and G2 showed significant reduction after TBBPA exposure. The animals also exhibited decreasing in body bends and changes in crawling trajectory adopted by worms showing a decrease). In general, body bendsand head thrashes were the most sensitive physiological indicators to evaluate the toxic effects of exposure to TBBPA (LIU *et al.*, 2020).

The survival rate of animals, although not altered in studies with different concentrations of TBBPA (0 to 200 µg L^{-1}) presents a change in their behavioral and physiological state. Exposure to 10 µg L^{-1} of TBBPA induced a decrease in body length (10–200 µg L^{-1}, $p < 0.05$). Changes in head thrashes were observed in worms exposedto TBBPA at 1-200 µg L^{-1} and the alterations magnified with increased concentrations($p < 0.05$). Pharyngeal pumping is an important toxicological marker since the functionality of the neural system that regulates its contraction is necessary for the ingestion of food by the animal. Exposure to TBBPA in the 100-200 µg L^{-1} designs promoted a decrease in the number of pharynx contractions ($p < 0.05$) (LIU *et al.*, 2020). The gene transcription profile of *C. elegans* is altered when exposed to TBBPA. 52 transcripts were down regulated and 105 were upregulated compared to the control group in qRT-PCR analyses (LIU *et al.*, 2020).

The chemical changes involved in chronic exposure to TBBPA in *C. elegans* involved the production of reactive oxygen species (ROS), in addition to superoxide dismutase

(SOD) and catalase (CAT). Worms exposed to TBBPA showed a significant increase in ROS in exposure to 1 µg L^{-1} of TBBPA (1–10 µg L^{-1}, $p < 0.05$; 100 µg L^{-1}, $p < 0.01$). In general, some pollutants have the ability to cause increased ROS levels in different model organisms. Exposure to 10 and 100 µg L^{-1} of TBBPA caused effects on SOD activity ($p < 0.01$) and CAT activity obeys a dose-response ratio showing increased activity in response to increased TBBPA concentrations (LIU *et al.*, 2019).

Chemical stress in *C. elegans* exposed to TBBPA promoted increased expression of stress-related genes. mRNA levels showed a decrease in *wah*-1, *ttr*-1, *mtl*-2, *gpd*-1, *mlc*-1, *mlc*-2 and increased of genes *ced*-1, *sod*-1, *sod*-3, *wnk*-1, M162.5, *ape*-1, *mtl*-1, *ctl*-1,*ctl*-2, *ctl*-3, *hsp*-16.1, *cyp*-35a2, and *hsp*-90 (Table 3) (LIU *et al.*, 2019).

Table 3. Gene expression profile in animals exposed to TBBPA, their respective molecular functions andin the development of *C. elegans*.

Expression genes	Biological functions
***wah*-1**	Involved in apoptotic DNA fragmentation, positive regulation of phosphatidylserine. Located in cytosol, mitochondria and nucleus (TROULINAKI *et al.*, 2018).
***ttr*-1**	Involved in the development process of animals, significantly increasing the generation of Dauer (HANSEN *et al.*, 2005).
***mtl*-1 and *mtl*-2**	Involved in response to heat and response to metal ions. The protein generated binds cations of several transition elements (FREEDMAN *et al.*, 1993).
***gpd*-1**	Involved in glycolytic process. Predicted to enable glyceraldehyde-3-phosphate dehydrogenase (NAD+) (phosphorylating) activity (HUANG *et al.*, 1989).
***mlc*-1, *mlc*-2**	Involved in several processes, including muscle organ development, nematode larval development and regulation of muscle contraction (SCHOOR *et al.*, 2022).

***ced*-1**	Involved in several processes, including cytoskeleton organization; left/right axis specification; and phagocytosis (ZHOU; HARTWIEG; HORVITZ, 2001).
***sod*-1 and *sod*-3**	Encodes superoxide dismutase, protecting against oxidative stress ensuring a normal lifespan (ROH *et al.*, 2009).
***ctl*-1, *ctl*-2 and *ctl*-3**	Encodes proteins with catalase and peroxidase activities protecting cells from ROS damage (AUGUSTI *et al.*, 2017).
***wnk*-1**	Predicted to be involved in hyperosmotic response (HISAMOTO *et al.*, 2008)
M162.5	Predicted to enable transmembrane transporter activity and involved in transmembrane transport.
***ape*-1**	Enables *p53* binding activity. Involved in negative regulation of apoptotic process (SCHLOTTERER *et al.*, 2010).
***hsp*-16.1**	Related to the stress response (LIN *et al.*, 2019).
***cyp*-35a2**	It reduces the fat content and is expressed in the intestine (AARNIO *et al.*, 2011).
***hsp*-90**	Involved in several processes, including positive regulation of phosphoprotein phosphatase activity, protein export from nucleus and protein stabilization (HIM *et al.*, 2009).

4.3 Microplastics

Currently, society benefits from using petroleum-derived products, such as plastic, since these materials provide stiffness and toughness, ductility, corrosion resistance, bio-inertness, high thermal/electrical insulation, and outstanding durability. Plastics are synthetic organic polymers obtained through the polymerization of monomers extracted from gaseous or oily matrices (COLE *et al.*, 2011). Despite being considered biologically inert and non-toxic, the years of use have demonstrated the huge impact of plastic disposal on the environment. The long half-life and the crescent amounts that are discarded in the environment can cause the

death of millions of animals yearly. In recent years, the additional concern regarding the formation of microplastics in the rivers and oceans has intensified since the natural process of plastic decomposition produces large amounts of tiny particles dispersed in the environment (THOMPSON *et al*., 2004). The dispersion of the pollutant allows its ingestion by various organisms, leading to various dysfunctions at the trophic level, as well as compromised metabolism and development (MILLER *et al*., 2020; THOMPSON *et al*., 2004).

Small plastic particles are defined as microplastics (MPs) when their dimension is greater than <5 mm (ROSAL, 2021). Exposure of organisms to MPs can cause several systemic toxic effects, with neurotoxicity being the major damage observed in several organisms. For instance, transgenerational effects are also observed in exposed organisms, and dysfunctions in egg-laying, body size, and movement in the experimental model *C. elegans* (CHEN *et al*., 2021). The size of the plastic particles are important for the ingestion and absorption process by the worms, with microplastic particles at 1 µm being potentially more harmful, increasing the toxic effects and lethality in nematodes compared to other sizes (LEI *et al*., 2018), given that the mouth size of *C. elegans* is 1.2 ± 0.03 µm in L1 larvae, 1.6 ± 0.1 µm in L2 larvae, 2.3 ± 0.1 µm in L3 larvae, and 4.1 ± 0.1 µm in young adults (DE; SAHU; SINGH, 2018).

Locomotion is one of the notable parameters in *C. elegans* important for observing neuronal viability since the nervous system must be intact to perform some activities. The worms showed decreased head shakes and body curvatures when exposed for a prolonged time to MPs, indicating toxic effects on the nervous system. D-type GABAergic motor neurons showed gap formation in exposed worms' ventral and dorsal cords, and *sod-3* and *acs-22* mutants presented higher susceptibility to neurotoxicity and underdevelopment of D-type motor neurons, indicative of neurodegeneration and damage to neuronal development. RNAi knockdown animalsfor *lgg-1* exposed to MPs exhibit severe damage to the ventral and dorsal cord and neuronal loss, given their crucial role in the autophagy process, given that NPs increase their level of expression (QU *et al*., 2019).

Defecation cycle is another important behavioral parameter for evaluating the

neurotoxicological effects of different materials. Exposure to MPs culminates in changes in defecation pattern (48.0 ± 1.2 s) after a 72 h exposure with MPs. Gene changes related to the defecation process and time of life were also changed. The expression of *skn-1* was almost significantly reduced, in addition to a decrease in *pmk-1*, and an increase in *mkk-4*, *cpr-1*, *itr-1* following exposure to PMs (5 μm) (SHANG *et al.*, 2020). The gene *skn-1* is directly related in detoxification and lifespan regulation, the *pmk-1* and *mkk-4* are involved in the intestinal intercellular MAPK signaling pathway, *cpr-1* and *itr-1* regulate the function of the foregut and hindgut, respectively.

Factors such as UV irradiation are essential in accelerating the toxic effects of MPs (LIU *et al.*, 2019). The natural aging of MPs exposure to UV alters their physicochemical properties, and studies indicate direct influences on their aging process induced by UV modify the characteristics and adsorption abilities through the oxidation process (XIONG *et al.*, 2020; LIU *et al.*, 2019). The changes cause increased toxicity in groupers (*Epinephelus Moara*) compared to the control (WANG *et al.*, 2020). In this context, chronic exposure to 10-100 μg L^{-1} of non-UV exposed polystyrene microplastics (PS-MPs) decreased the frequency of head spasms in *C. elegans* animals compared to control. However, chronic exposure to a lower concentration (1 μg L^{-1}) of UV-exposed PS-MPs significantly decreased the rate of head trashes. The same occurred with body curvature, where animals treated with PS-MPs exposed to UV showed a significant decrease in this behavior (CHEN *et al.*, 2021). Morphological changes in dopaminergic, glutamatergic and serotonergic neurons occurred after exposure to PS-MPs, where chronic exposure to 10-100 μg L^{-1} UV exposed significantly decreased the fluorescence intensity of glutamatergic and dopaminergic neurons in GFP-tagged transgenic strains. Exposure to 1-100 μg L^{-1} influenced the fluorescence intensity in serotonergic neurons compared to control, being exacerbated by the treatment with UV-exposed PS-MPs (CHEN *et al.*, 2021).

4.4 Graphene Oxide

Graphene oxide (GO) is one of the oxidized forms of graphene and a new carbon-based two-dimensional engineering nanomaterial (ENM) (DERAKHSHI; DAEMI; SHAHINI, 2022).

Thanks to its physical compound properties, GO can potentially be used in biosensors, bioimaging, drug delivery and environmental remediation (MAGNE *et al.*, 2021). Graphene, graphene oxide, and treated graphene oxide are considered promising candidates for industrial, and biomedical applications due to their exceptionally high rigidity and mechanical strength, excellent electrical conductivity, high optical transparency, and good biocompatibility (LIAO; LI; TJONG, 2018). Despite the enormous economic benefits of GO to humanity, the increase in its production and environmental emissions have also brought environmental risks. This has called attention in toxicological studies (JIN *et al.*, 2022).

In vitro studies have confirmed damage caused by GO in mammals and observed GO accumulation inside cells affecting morphology, function, viability, mortality, and membrane integrity of human cells (JIN *et al.*, 2022).

Recent studies on GO exposure demonstrate that there is an induction of toxicity, including the production of high levels of ROS, cellular apoptosis, and inflammation *in vitro* and *in vivo*. It suggests that exposure to these particles may cause some human health problems. Because of this, precautions have been taken with GO, and several toxicological studies have been performed in various animal models to show possible health side effects caused by nanomaterials. Some studies have been carried out with nematodes showing that exposure to GO can induce reproductive toxicity and immunotoxicity. Despite these significant concerns, little is known about its adverse effects on the nervous system, including neuronal damage or degeneration (GHAZIMORADI; GHORBANI; EBADIAN, 2022).

GO is widely distributed and can enter organisms and cross a blood-brain barrier, reaching the central nervous system, promoting certain neurotoxicity and high neuronal accumulation, causing degeneration and brain necrosis (BOYES; VAN THRIEL, 2020).

Recent *in vivo* studies have shown graphene-derived nanomaterials, including GO, can enter living organisms by inhalation, ingestion, skin penetration, or injection, distributing mainly in vascularized organs such as kidney, spleen, liver, lung and other tissues and organs (JIN *et al.,* 2022).

In *C. elegans* GO can be observed throughout the body and in the anterior part of the intestine with the aid of fluorescent labeling. The translocation of GO in intestinal worm cells has a distribution adjacent or surrounding the mitochondria and can cause adverse effects on the nematode by influencing the development or function of mitochondria, such as the formation of reactive species and mitochondrial respiration (WU *et al.*, 2013; LEUNG *et al.*, 2008).

Furthermore, the graphene derivative can be translocated and distributed in different organs or tissues, and therefore, bioavailability plays a crucial role in inducing its toxicity. Previous reports show that exposure to GO nanosheets induces oxidative stress and that oxidative stress has been widely accepted as one of the components of GO neurotoxicity (TABISH *et al.*, 2018).

C. elegans, a free-living nematode with important ecological significance in soil nutrient cycling and which has conserved basic physiological processes, signal transduction pathways, and epigenetic markers with humans, allows the comparison of corresponding molecular mechanisms (WU *et al.*, 2013).

Therefore, it is one of the main models proposed to investigate the neurotoxic effect of GO (LEUNG *et al.*, 2008). As a result, evaluations with *C. elegans* indicate that prolonged exposure to GO in L4 larvae can potentially cause adverse effects such as significant reduction in litter size, body length, decreased locomotion behavior,induction of intestinal autofluorescence due to lysosomal deposits of lipofuscin, and excessive production of reactive oxygen species (ROS) (WU *et al.*, 2013).These results suggest that prolonged GO exposure may impair primary and secondary target organ functions in *C. elegans*. When examining the molecular and epigenetic basis of exposure to GO, it has been observed that prolonged exposure to GO alters the expression pattern of genes necessary for oxidative stress. The prolonged exposure to GO caused changes in the expression level of genes such as *sod-1*, *sod-4* encodes copper/zinc superoxide dismutase, *sod-2*, and *sod-3* encode manganese superoxide dismutase, and *isp-1* encodes an iron-sulfur protein." Rieske", among others (WU *et al.*, 2013).

Other findings indicate that exposure to GO induces an increase in ROS levels, as well as damage to neuronal systems, such as dopaminergic and serotonergic

systems. In addition, some critical pathways of the worm in the regulation of the immune system in response to bacterial bacteria (Wnt/β-catenin BAR-1) were found to be increased by exposure to GO in nematodes (LIU *et al.,* 2020).

4.5 Metals

Metals are generally divided into two groups: essential and nonessential metals. Essential metals include copper, iron, manganese, nickel, and zinc, which will be highlighted here. They are persistent environmental contaminants indispensable to life and play an essential role in brain metabolic processes. However, the occurrence of metal dysregulation in the brain has been described in the literature as being associated with neuronal damage and the triggering of neurodegenerative disorders (SOARES; FAGUNDES; AVILA, 2017).

The potent redox activity of copper is required for sustaining life (COTRUVO *et al.*, 2015). Conversely, in *C. elegans*, the excessive copper levels cause neurotoxic effects associated with locomotor behavioral disorders in a dose and time-dependent manner (COTRUVO *e al.*, 2015; LI *et al.*, 2017).

Copper neurotoxicity induces spatial memory impairment *in vivo* (CERPA *et al.*, 2004), a behavior studied in the nematodes. Thus, memory damage related to copper neurotoxicity can be evaluated using an AD model in *C. elegans* (CL2006 strain), which has been widely used to enhance the understanding of molecular pathogenesis and to identify promising therapeutic strategies (DU *et al.*, 2019).

In this context, elevated amounts of metals, such as copper, play an important role in the pathogenesis of AD by inducing aggregation, protein misfolding, and generation of ROS (SU *et al.*, 2020).Accumulated copper causes neuronal damage and aggravates the aggregation and phenotypical changes caused by mutated Amyloid beta peptide 42 (Aβ42) in *C. elegans* (CL2020 strain) (SU *et al.*, 2020).

One of the major potential sources of free radical production in the brain is transition metals like copper and iron (BUSH, 2000). ROS generated by iron damages the cholinergic system, interrupting the communication between motor neurons and altering the worms' acetylcholinesterase activity (CARVALHO *et al.*, 2021). Iron-

neurotoxicity in *C. elegans* also includes dopaminergic neurons (FAGUNDES *et al.*, 2015); an increase in iron levels can increase dopamine synthesis, causing excessive dopamine to be released into the cytoplasm, which may lead to increased ROS production (CHEGE; MCCOLL, 2014). Besides, it is important to note that several iron compounds (such as iron chelates, zerovalent iron nanoparticles, or iron salts) havebeen recognized to be neurotoxic in *C. elegans* (CARVALHO *et al.*, 2021).

Regarding the manganese neurotoxicity in nematodes, it is well established that dopaminergic neurons are involved (WITTKOWSKI *et al.*, 2019). Since dopamine release at the synapse is essential to induce manganese-associated neurodegeneration (MARTINEZ-FINLEY *et al.*, 2013). Consequently, exposed subjects may develop a syndrome known as manganism, characterized by Parkinson's disease-like symptoms such as alterations in movement, speech, and facial expression (SOARES *et al.*, 2020). Manganese exposure modulates heat shock protein (HSP) expression, particularly HSP-70; a decrease of HSP-70 results in increased protein oxidation and increased dopaminergic neurodegeneration in worms (AVILA *et al.*, 2016). Moreover, the influence of manganese neurotoxic effects in the cholinergic, GABAergic and glutamatergic systems seems very low or does not occur (CHAKRABORTY;ASCHNER, 2012). In addition, a brief sub-lethal manganese exposure to *C. elegans* increases ROS production and decreases mitochondrial membrane potential and oxygen consumption (SETTIVARI; LEVORA; NASS, 2009). In this sense, altered manganese homeostasis induced dopaminergic neurodegeneration associated with over-expression of the important transcription factor SKN-1 (NRF2 in humans) in *C. elegans* (CHAKRABORTY; ASCHNER, 2012). Manganese-treated worms exhibit deficits in locomotor function (LEYVA-ILLADES *et al.*, 2014), similar to humans' neuromotor consequences of manganese toxicity (TAYLOR *et al.*, 2020). In addition, repeated manganese exposure alters adaptive developmental plasticity causing remodeling in its innate learning behavior(RAI; NAIR, 2021). Neurodegeneration is evident in *C. elegans* when exposed to Manganese, as L1 stage worms present damageto DAergic neurons after exposure to Mn (CHAKRABORTY *et al.*, 2015). Worms in theL4 stage do not show reduction and changes in the viability of DAergic neurons, suggesting the susceptibility of younger animals to exposure to Mn (GUBERT *et al.*, 2018).

About nickel, a morphological study with GFP-expressing worms showed significantly increased degeneration of cholinergic, dopaminergic, and GABAergic neurons with increasing nickel concentration (IJOMONE *et al.*, 2020). Oxidative stress may be associated with neuronal damage (cholinergic, dopaminergic, and GABAergic neurons) and altered locomotor behavior after nickel exposure during worm development (IJOMONE *et al.*, 2020), this locomotor behavior reflects the neuronal status in *C. elegans* (LEUNG *et al.*, 2008*)*. Few existing studies have closely examined the neurotoxicity of nickel in *C. elegans.*

Evidence shows that Aβ42 transgenic *C. elegans* presented neurotoxicity involving ROS production and paralysis induced by zinc administration (XIE *et al.,* 2021). Although zinc is considered a redox-inert metal ion, there is still evidence that zinc plays an increase in oxidative stress. Besides, exposure to mixtures of zinc, copper, and/or cadmium harm locomotion behavior in worms; these effects could involve motor neuron dysregulation (MOYSON *et al.*, 2018; WANG; SHEN. WANG, 2007). Little is known about zinc neurotoxicity in *C. elegans*.

Additionally, nonessential metals can also provoke neurotoxicity in *C. elegans:* the following are some of the nonessential metals and their possible neurotoxic effects. Aluminum exposure causes a decrease in glutamate, serotonin, and dopamine levels, associated with behavioral deficits (LI *et al.*, 2013). In addition, acute exposure of worms to cadmium can induce neurodegeneration in dopaminergic neurons once it was observed a significant reduction in GFP signal intensity in dopaminergic neurons following cadmium treatment (LAWES *et al.*, 2020). Lead exposure induces dopaminergic neurotoxicity in *C. elegans* because lead treatment affects dopaminergic cell morphology and structure in worms expressing GFP under a dopaminergic cell-specific promoter as well as alters the dopamine transporter (*dat-1*) gene level (AKINYEMI *et al.*, 2019). Another study showed that morphological changes in AFD neurons were observed after lead treatment; a decrease in locomotor assays could be attributed to changes in AFD neuron function as well as an increase in the oxidative status in *C. elegans* (YU; LIAO, 2014). Another metal that affects AFD neurons is arsenic, following early-life exposure. This damage was associated with increased ROS production, followed by neurobehavioral changes, like impaired thermosensory

function. The AFD neurons are two ciliated neurons responsible for various functions related to thermotactic behaviors and interact with the AIY interneuron.

Moreover, the involvement of the *abts-1* (bicarbonate transporter) in arsenic developmental neurotoxicity has been demonstrated because *abts-1* expression was increased in the neurons of transgenic worms. Furthermore, mercury, especially its organic form – methylmercury, is one of the most poisonous neurotoxicants found in the environment. Mercury exposure induces degeneration of GABAergic neurons; of importance, younger larvae (L1-L3) showed greater sensitivity to neurotoxicity (assessed by changes in neuronal survival and synaptic function (XING *et al.*, 2009).

Acute early-life exposure causes DAergic neurodegeneration later in life and a decrease in dopamine levels of nematodes, suggesting that the dopamine system is particularly susceptible to methylmercury (MARTINEZ-FINLEY *et al.*, 2013a).

To date, numerous neurobehavioral studies have confirmed methylmercury-induced neurodevelopmental toxicity in young *C. elegans* (MARTINEZ-FINLEY *et al.*, 2013a; MCELWEE; FREEDMAN, 2011), such as the significant decrease in associative learning behavior.

6. Conclusion

The organism *C. elegans*, among its wide applicability, is an essential model for studies of environmental pollutants and prospection of its exposure's possible biological and molecular effects. Its nervous system, with the totality of the mapping of neural networks, connectivity, and neurotransmitters, guarantees and makes viable *in vivo* neurobehavioral observations, as well as damage at the cellular level in different types of neurons with biological characteristics comparable to human cellular structures. The behavioral dysregulations promoted by exposure to neurotoxic agents, such as an imbalance in the locomotor, food, and reproductive profile, enable the observation at a macroscopic level of biochemical and particular changes in muscle cells, motor and sensory neurons, regulated by different chemical signals induced by neurotransmitters that act in regulatory, excitatory and inhibitory systems. In general, other possibilities of neurotoxic staging are promoted by different synthetic and natural chemical compounds, in addition to promoting the experimental model *C. elegans* in the toxic

evaluation of these compounds.

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Abbreviations

TBBPA - Tetrabromobisphenol A
GO - Graphene Oxide
MPs - Microplastics
PS-MPs - Polystyrene microplastic
ACh - Acetylcholine
Glu - Glutamate
GABA - γ-amino butyric acid
DA - Dopamine
5-HT - Serotonine
GFP - Green fluorescent protein
YFP - Yellow fluorescent protein
mCherry - mCherry fluorescent protein
AD - Alzheimer's disease
PD - Parkinson's disease
QP - Quinalphos
MCP -Monocrotophos
ATP - Adenosine triphosphate
CMs - Carbamate
OPs - Organophosphorus
CAR - Carbofuran
MET - Methomyl
CPF - Chlorpyrifos
TAP - Triazophos
LDH - Lactate dehydrogenase
ROS - Reactive oxygen species
SOD - Superoxide dismutase
CAT - Catalase
Aβ42 - Amyloid beta peptide
HSP - Heat shock protein

Chapter 2

C. elegans in cancer research

Aline Castro Silva
Maria Eduarda Oliveira de Souza
Flávia Suelen de Oliveira Pereira
Heloísa Aiolfi Padilha
Andy Joel Taipe Huisa
Daiana Silva Ávila

Abstract

Cancer is characterized by the uncontrolled growth of cells that can invade tissues causing metastasis. Cancer cells have specific characteristics such as uncontrolled proliferation, differentiation, loss of function and resistance to cellular homeostasis mechanisms, making them highly capable of invading other tissues. The nematode *Caenorhabditis elegans* provides advances in the understanding of cancer progression, as its homology with human genes and the possibility of obtaining mutant strains allow the application of the model to understand a range of diseases, including tumorigenesis. Among the pathways known to be involved in the formation of tumors in humans are Ras, Wnt and Notch, in addition to the ESPL1, FOS and p53/BRCA-1 genes. In *C. elegans*, mutations such as inhibition or overexpression of these signaling pathways or genes can cause different physiological changes in the worm recognized as different phenotypes. In this chapter, we will discuss the main pathways and genes involved in the formation of tumors in mammals and *C. elegans*, in addition to the use of the alternative experimental model for the screening of new molecules with anticancer potential.

1. Introduction

Cancer comprises a group of diseases related to the uncontrolled growth of cells that tend to invade tissues and spread to other parts of the body. Several characteristics in cancer cells confer advantages for tumor formation and dissemination, such as uncontrolled proliferation, differentiation and loss of function, resistance to cell death, genome instability, angiogenesis induction, invasiveness, and the ability to undergo metastases (Kyriakakis, Markaki and Tavernarakis, 2015).

Thus, it is necessary to understand the main mechanisms involved in these oncogenic diseases in order to evolve with the development of methods to combat or

prevent the growth of tumors. In addition, due to its complexity, cancer treatment is known to be much more effective when performed in the early stages. Therefore, it is also essential to develop detection methods for an early diagnosis (Hirotsu *et al.*, 2015).

Several alternative models have been used in cancer-related studies, including worms, which were pioneers in helping to discover relevant mechanisms to tumors in humans. Studies using the nematode *Caenorhabditis elegans* provided advances in the understanding of cancer progression, especially in the processes involved in cell cycle progression, invasion, and metastasis (Kirienko, Mani and Fay, 2010; Hirotsu *et al.*, 2015).

The invertebrate *C. elegans* has many advantages such as small size, easy handling and maintenance, rapid reproduction, short life cycle, transparent body, which is essential for analyzes with fluorescent markers, a completely sequenced genome and with about 60-80% of the homologous genes in humans, in addition to the availability of thousands of mutant strains that allow the application of the model to comprehend a range of diseases. Therefore, it is an interesting option to explore issues such as evolution, development, cell fate specification, stem cell regulation, tumorigenesis and aging (Kobet *et al.*, 2014).

According to the literature, mutations in signaling pathways, including the Wnt, Notch, and Ras-ERK signaling cascades, may be a mechanism for the development of cancer in humans (Kobet *et al.*, 2014). *C. elegans* presents orthologous to these genes and mutations in the wild-type strain can alter cell division and induce hyperplasia. That said, the relevance of using *C. elegans* as a model of studies for cancer is evident, since these pathways are related to the development of the worm germ line, making it possible to assess issues such as cell proliferation, cell differentiation, progression of cell cycle, fate, and death (Medina, Ponce and Cruz, 2021). In addition, *C. elegans* is the model of choice in many studies, as it respects the principle of the 3Rs (replacement, reduction and refinement) and is being used in cancer research for the development of methodologies for the early detection of the disease (Hirotsu *et al.*, 2015) and for initial screenings of safety and efficacy of new anticancer drugs (Wellenberg *et al.*, 2021), such as itraconazole, disulfiram, etodolac and ouabain

evaluated by Medina et al. (2021) in mutant strains of *C. elegans* such as Wnt (JK3476), Notch (JK1107 and BS3164) and Ras-ERK (SD939 and MT2124).

2. Main pathways and genes involved in cancer development in *C. elegans*

Due to its well-characterized genetic sequencing and developmental lineage, the *C. elegans* is a model that is increasingly encompassing studies of evolution, development, cell fate specification, stem cell regulation, aging and, currently, tumorigenesis. Current data show that about 52.6% of human genes, encoding proteins, have orthologs in the nematode, that is, genes and signaling pathways remain conserved, facilitating the study of human diseases and the identification of therapeutic targets. Mutations such as the inhibition or overexpression of these signaling pathways or genes can cause various physiological changes in the worm recognized as different phenotypes, such as: the loss or uncontrolled proliferation of distal tip somatic cells and germline stem cells, extra DTC (distal tip cell) formation, abnormality of germ cell fate specification, changes being associated with the phenotype of sterility, infertility, and germline tumor formation (Corsi, Wightman and Chalfie, 2015; KIM, Woojin et al.).

We will describe in this topic the main signaling pathways and oncogenes, their biological functions, the formation of cancer cells and the conservation of the pathways in *C. elegans* and in mammals (Kirienko, Mani and Fay, 2010).

2.1 RAS/RAF/MEK/ERK signaling pathway

The RAS/RAF/MEK/ERK intracellular signaling pathway or ERK/MAPK pathway, conserved in all eukaryotes, acts in several biological processes essential for survival such as cell differentiation, apoptosis, proliferation, homeostasis, and angiogenesis. However, it is already known that this pathway is related to tumorigenesis and metastasis. Its activation process in the extracellular environment begins when there is a stimulus in the receptor, of factors such as: virus, endothelial growth factor (EGF), tumor necrosis factor alpha (TNF-α), cytokines, protein-coupled receptor ligands G, and oncogenes. These can stimulate the pathway in at least four different ways through: activation through protein kinase C (PCK), activation of the

Ras receptor tyrosine kinase, activation of the G protein-coupled receptor, or Ca^{2+} activation (Kobet *et al.*, 2014; Guo *et al.*, 2020).

In the cytoplasm, after the interaction of the ligand with the tyrosine kinase receptor or with the endothelial growth factor receptor (EGFR), the Grb2 (Growth Factor Receptor-Bound Protein 2) adapter protein will be activated, binding to the receptor and the guanine nucleotide exchange factor, SOS (Pro-rich domain of the GTP-GDP exchange factor). The formed Grb2/SOS complex, coupled to the phosphorylated receptor, promotes the activation of the next protein in the cascade, Ras protein (Da Silva PH, 2017).

Encoded by the *Ha-ras*, *Hi-ras*, and *N-ras* genes, the Ras G protein is activated when it is in a GTP-bound conformation and becomes inactive when bound to GDP.Ras will continue the pathway by translocating to the cell membrane, phosphorylating and activating the protein kinase Raf. The Raf protein, encoded by the *raf* gene, after binding to Ras, acts as a serine-threonine kinase. One of its three subtypes, Raf-1, is active in the signal transduction pathway, which in sequence will phosphorylate and activate MEK (MAPK activating kinase). Consequently, as shown in figure 1, MEK will also perform the phosphorylation and dimerization of ERK or MAPK (Mitogen Activated Protein Kinase) (Silva *et al.*, 2009; Garavello *et al.*, 2013; Guo *et al.*, 2020). The last step of the ERK/MAPK pathway is considered the main one. If altered (hyper- stimulated or inhibited), it will directly affect the cellular signal transduction network. This protein also belongs to the serine/threonine-protein kinase family, which, when inactive, is anchored in the cytoplasm by MEK, and, when activated, is translocated to the cell nucleus regulating gene expression and transcription factor activity. This process will cause signs of cell division, differentiation, development, and migration, in addition to having anti-apoptotic action, one of the hallmarks of tumor cells (Kobet *et al.*, 2014; Guo *et al.*, 2020).

Therefore, this pathway is not only related to biological functions but also closely linked to tumor formation. An example is when ERK expression is high, being detected in these cases in human cancers, such as ovarian, colon, breast, and lung. Another example is when receptor overexpression and Ras protein mutation occur (Roberts and Der, 2007; Guo *et al.*, 2020).

As already mentioned, this is one of the biological processes that remains conserved in all eukaryotes, having protein homologs involved in the *C. elegans* germ line. The pathway remains with the same steps, except for the protein names, being them: LET-23 (EGRF homologue), which will activate LET-60 (Ras homologue), followed by the stimulation of LIN-45 (Raf homologue), downstream to MEK-2 (homologue of MEK), and ending the cascade with MPK-1 (homologue of ERK). In the nematode, the Ras/Raf/MEK/ERK pathway is involved with the specification of sperm fate, apoptosis, oocyte activation, vulvar development, spermatogenesis, meiotic cycle progression, and axon orientation. In table 1, the main genes of the worm that express the proteins of the signaling pathway are listed, among them, *let-60* (human Ras ortholog). The overexpression of *let-60* results in the formation of ectopic pseudovulvas that have no function, characterized by the multivulva (Muv) phenotype, in addition to the hatching of larvae inside the worm. Other null mutants for *lin-45*, *mek-2*, or *mpk-1* show a phenotype of sterility in adulthood, as well as the *ksr-2* (kinase suppressor) mutant. All strains exhibit arrests in the progression of the pachytene stage of prophase in the cell meiosis process in the germline. Recent studies have used the *C. elegans* transgenic cancer models for the screening of new anticancer drugs (Ohmachi *et al.*, 2002; Guo *et al.*, 2020). The exposure *let-60* overexpressed worms to some drugs, which have been shown cytotoxic activity in studies, showed a decrease worms that developed the multivulva phenotype, dependent on concentration, among them the most significant results were from Etodolac and Itraconazole (Medina, Ponce and Cruz, 2021). It is already known that these molecules act by activating cellular apoptosis among other functions related to carcinogenesis.

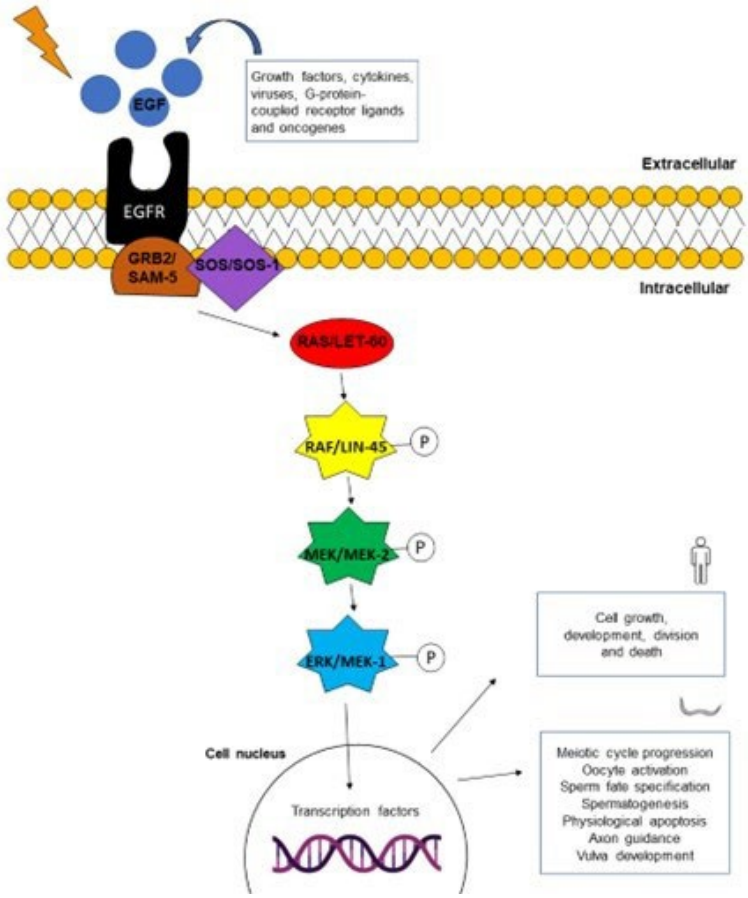

Figure 1. RAS/RAF/MEK/ERK signaling pathway. Comparison between human proteins and homologous nematode proteins, from receptor stimulation, protein phosphorylation cascade, to activation of transcription factors in the cell nucleus. EGF: endothelial growth factor; EGFR: endothelial growth factor receptor; GRB2: Growth Factor Receptor-Bound Protein 2; MEK: mitogen-activated protein kinase. Modified from Kobet *et al.*, 2014.

2.2 ESPL1

The *espl-1* gene is a cysteine separase-1 protease transcription factor, belonging to the same class as caspases, which are pro-apoptotic enzymes. In mammals, it is essential for chromosomal segregation, regulates the trafic of substances through the membrane in the anaphase phase of meiosis and cell mitosis, in apoptosis and centriole duplication, also interfering in other stages of the cell cycle (Melesse *et al.*, 2018; Cotterill SJ, 2019; Jeong *et al.*, 2020). The gene is highly conserved in *C. elegans* whose roles involve the meiotic anaphase phase, promotion of cortical granule exocytosis after fertilization, releasing of proteins that contribute to the eggshell formation. In mammals, it is involved in the extracellular cover of the zygote, providing protection to the fertilized embryo. Therefore, exocytosis depends on the action of this separase,

which in the nematode is the ortholog named *sep-1*. In addition to this function, *sep-1* has other similar physiological actions that interfere in the process of cell division of somatic cells and the germline, in gametogenesis and programmed cell death (Bembenek *et al.*, 2007; Jeong *et al.*, 2020).

Despite being recently discovered and still little explored, researchers are already seeking for *sep-1* mutants in the nematode to explore and elucidate its function and regulation in the organism. In the work of (Melesse *et al.*, 2018) they have observed that *sep-1* suppressor mutants mostly present embryonic lethality, formation of an impermeable eggshell, chromosomal disjunction and failures in cytokinesis due to its defective action on cell division. The *sep-1(e2046)* mutant showed sensitivity to temperature, being viable only at 15°C. With RNAi gene silencing, however, extensive failures in chromosome segregation were obtained, compared to the *sep-1(e2406)* allele, which had minimal effects.

These defects in chromosome segregation can lead to several genetic syndromes, associated with the growth and progression of different types of tumor cells, therefore the activity of *espl-1* still needs to be further investigated (Jeong *et al.*, 2020).

2.3 FOS

It is already known that the human c-Fos gene encodes the transcription factor AP-1 (activator protein-1), which regulates the expression of several genes, including some that interfere with the growth and development of cancer cells (Oliveira-Ferrer *et al.*, 2014). As already mentioned, there are some cell signaling pathways that, when suffering some change, can directly contribute to the formation of different types of tumors. The Ras/Raf/MEK/ERK and Notch pathways are related to the activation of numerous factors, including the nuclear transcription factor c-Fos, which has been increasingly related to the development of tumors (Medwig-Kinney *et al.*, 2019; Guo *et al.*, 2020). Thus, some studiesdemonstrate the role of c-Fos as an oncogene, being correlated with the progression and detection of bone, skin, brain, pancreas, and ovarian cancer, in addition to non-small cell lung cancer (NSCLCs), sarcomas, among others that are being studied (Oliveira-Ferrer *et al.*, 2014; Guo *et al.*, 2015; Abarrategi *et al.*, 2018; Manios *et al.*,

2020).

c-Fos gene is being targeted as a prognostic marker of these cancers and for new therapies and anticancer agents. In addition to its possible activity as a tumor suppressor in cases of thyroid tumors and the progression of liver cancer, it may be associated with the prognosis of gastric, ovarian, and pancreatic cancer. As a treatment, the association of a c-Fos inhibitor (T-5254) with eribulin (anticancer agent) shows a strong effect in blocking the proliferation of tumor cells (MDAMB-231 and HCC70) and in a mouse breast cancer tumor model, proving that the high expression of Fos in these cells decreased the sensitivity to eribulin (Oliveira-Ferrer *et al.*, 2014;Guo *et al.*, 2015; Tanaka *et al.*, 2020).

Since studies using this gene have become crucial to screen new drugs in different models, the nematode *C. elegans* also has benefits to contribute in such a field. Its ortholog in the worm is *fos-1,* whose main physiological action is to regulate uterine and vulva development, and control ovulation. In *fos-1* mutants, the phenotype comprises blockage in the oocyte fertilization process and ovulation defect (Hiatt *et al.*, 2009). Moreover, it has been evidenced by its relationship with KGB-1 (GLH-Binding kinase 1), a stress-activated kinase. *fos-1* positively regulates its signaling, triggering protective functions against heavy metals and resistance to stress independent of the age of the worm, directly affecting its lifespan (Zhang *et al.*, 2017).

2.4 Wnt signaling pathway

The human Wnt family is composed of nineteen different cysteine-glycoproteins acting as ligands for more than 15 receptors (Komiya and Habas, 2008; Yang *et al.*, 2016). This signaling pathway is intimately involved in stem cell differentiation, self-renewal, cell fate, and cell migration (Schambony and Wedlich, 2007; Nusse, 2008). Thus, dysfunctional Wnt signaling has been linked to the evolution of leukemic stem cells, and many other cancers (Duchartre, Kim and Kahn, 2016; Medina, Ponce and Cruz, 2021). There are basically three Wnt signaling sub-pathways (all activated by the binding of the Wnt ligand to the Frizzled Family receptor): i) canonical Wnt/β-catenin pathway: pathway studied in more detail, which controls the expression of specific target genes through the β-catenin effector protein (Angers and

Moon, 2009; Clevers and Nusse, 2012); ii) non-canonical Wnt/planar cell polarity (PCP) and; iii) non-canonical Wnt/calcium pathway. In mammals, the non-canonical pathways act independently of β-catenin (Lawrence, Struhl and Casal, 2007; Green, Inoue and Sternberg, 2008; James, Conrad and Moon, 2008; Angers and Moon, 2009). However, Wnt signaling involves the integration of these three sub-pathways, and allof them need to be considered to evaluate the effects of Wnt signaling modulation (Moon *et al.*, 2004; Florian *et al.*, 2013; Thrasivoulou, Millar and Ahmed, 2013).

2.4.1 The canonical pathway: Wnt/β-catenin

β-catenin is an unstable protein, whose cytoplasmatic concentration is controlled, and is encoded by the CTNNB1 gene in humans (Kobet *et al.*, 2014; Duchartre, Kim and Kahn, 2016). In the absence of Wnt ligands, cytoplasmic β-cateninis the target of a degradation complex formed by APC (Adenomatous polyposis coli) and Axin scaffold proteins, being then phosphorylated by CK-1 (casein kinase 1) and GSK-3 (Glycogen Synthase Kinase- 3) and further degraded by the proteasome (Figure 2A). Therefore, in the canonical pathway, CK-1, GSK-3, APC and Axin act as negative regulators. After activation (presence of Wnt ligands), the formation of the APC/Axin/CK-1/GSK-3 destruction complex is inhibited, which stabilizes β-catenin and leads to its localization in the nucleus (Figure 2B). In the nucleus, β-catenin interacts with transcription factors of the TCF family to activate the expression of target genes such as FGF20, DKK1, WISP1, MYC and Cyclin D1 (Eisenmann, 2005; Kobet *et al.*, 2014). The target genes of this pathway have been linked to the development of several types of cancer, including breast, ovarian, leukemia, and thyroid (Holland *et al.*, 2013; Yang *et al.*, 2016). The canonical Wnt/β-catenin is requiredto regulate embryonic stem cells, adult stem cells, and cancer stem cells. Furthermore, Wnt/β-catenin signaling plays a role in regulating cancer stem cells and stem cells in the nervous system, hematopoietic system, skin, and intestine (Holland *et al.*, 2013;Yang *et al.*, 2016). Thus, the inhibition of Wnt/β-catenin signaling may reduce the capacity of cancer stem cells, which may be of potential therapeutic benefit in the treatment of various types of cancer.

In mammals, β-catenin acts in cell adhesion and Wnt signaling. It acts on the actin cytoskeleton to adherens junctions and interacts with TCF/LEF-1 transcription factors to

activate expression of the Wnt target genes (Cadigan and Nusse, 1997; Clevers and Nusse, 2012). Like flies and vertebrates, *C. elegans* has several genes encoding Wnt ligands (*lin-44, egl-20, mom-2, cwn-1,* and *cwn-2*) (Thorpe *et al.*, 1997; Maloof *et al.*, 1999), Frizzled family Wnt receptors (*lin-17, mom-5, mig-1 and cfz-2*)(Ruvkun and Hobert, 1998; Zhao et al., 2003) *and* Disheveled proteins (*mig-5, dsh-1, dsh-2*) (Ruvkun and Hobert, 1998). However, unlike other species, *C. elegans* has four genes encoding divergent β-catenin proteins (*bar-1, wrm-1, sys-1* and *hmp-2*) (Rocheleau *et al.*, 1997; Costa *et al.*, 1998; Eisenmann *et al.*, 1998; Robertson and Lin, 2012). The β-catenin that mediates the canonical signaling of Wnt in *C. elegans* is BAR-1, the only β-catenin from *C. elegans* that acts directly with TAF/POP-1 (Herman, 2002; Eisenmann, 2005; Sawa and Korswagen, 2013). Evidence suggests that the canonicalWnt signaling pathway plays a role in four processes: the migration of neuroblasts; the specification of the fate of the six vulvar precursor cells (VPCs); posterior hypodermic P12 cells; and gene expression in posterior seam cells. All these pathways use BAR-1 β-catenin and target a Hox gene (essential for the development of the organismo) (Eisenmann, 2005).

In *C. elegans*, canonical Wnt/β-catenin signaling functions as the niche for germ stem cells known as distal tip cells (DTC), is essential for their maintenance (Kimble and Crittenden, 2007; Kobet *et al.*, 2014). During early larval development, Wnt/β-catenin signaling plays an essential role in DTC target specification in *C. elegans* gonads. Therefore weak or no Wnt signaling results in loss of DTC and consequently no germ-line stem cells, which partially or entirely cause sterility. *C. elegans* has several mutants that promote the study of the Wnt signaling pathway, including *pop-1(q645)* and *sys-1(q544)*, that present total loss of DTC and sterility (Siegfried and Kimble, 2002; Kidd *et al.*, 2005), *lin-17(n671)*, with loss of DTC (<10%) and partial sterility (Phillips *et al.*, 2007), *ceh-22(q632)* loss of DTC (~40%) and partial sterility (Lam, Chesney and Kimble, 2006), *hs::sys-1* extra DTC and fertility (Kidd *et al.*, 2005) and *hs::ceh-22,* that presents extra DTC and fertility (Lam, Chesney and Kimble, 2006) (Table 1).

Although it is not a specific biomarker for the Wnt pathway, the *lag-2*::GFP reporter gene can be used to visualize DTCs (Blelloch *et al.*, 1999; Kobet *et al.*, 2014;Lam and Phillips, 2017). Typically, wild-type hermaphrodites have two DTCs (Byrd *et al.*, 2014), and most *ceh-22* mutants lack both DTCs and are completely sterile. However, increased

expression of the *ceh-22* gene produces extra DTCs (Lam and Phillips, 2017). Thus, *ceh-22* (q632) loss of function mutant is an attractive allele for identifying drugs that can inhibit or activate the Wnt/β-catenin signaling pathway. In addition, studies indicate that DTC fate is also regulated by cell cycle regulators, asCyclin D is required for DTC target specification (Tetsu and McCormick, 1999; Tilmann and Kimble, 2005). Cell cycle regulators work in conjunction with Wnt/β- catenin signaling to specify the fate of the DTC (Lee *et al.*, 2014). Therefore, it suggests the possibility that the drugs may target cell cycle regulators and/or Wnt/β-catenin signaling.

2.4.2 The non-canonical pathways: Wnt/planar cell polarity (PCP) and Wnt/calcium

In Drosophila and vertebrates, Wnt signaling pathways that do not include a β- catenin homolog have been termed "non-canonical" (Herman, 2002). They actindependently of β-catenin and are more generally associated with cell differentiation, polarity, and migration (Duchartre, Kim and Kahn, 2016) (Lai et al., 2009).

In the Wnt/planar cell polarity (PCP) pathway, Wnt ligands can bind to Frizzled receptors and activate small GTPases, such as RhoA (Ras homolog gene family member A), RAC (Ras-related C3 botulinum toxin substrate) and Cdc42 (cell division control protein 42) (Lai, Chien and Moon, 2009). The PCP pathway affects the cytoskeleton and triggers transcriptional activation of target genes responsible for cell adhesion and migration (Yamamoto *et al.*, 2008). In the calcium-dependent Wnt pathway, Wnt ligands use Frizzled and RYK or ROR receptors (alternative receptors) increasing cell migration and inhibiting the canonical Wnt/β-catenin pathway, managing intracellular calcium flux and activating calmodulin kinase II (CaMK2), Jun kinase (JNK) and PKC (Eisenmann, 2005).

In *C. elegans* the non-canonical Wnt pathway is still not fully understood (Sawa and Korswagen, 2013). The non-canonical pathway uses β-catenin WRM-1, as it acts weakly with POP-1 (Sawa and Korswagen, 2013) and resulting in the loss of DTC. HMP-2 does not interact with POP-1 but is the only β-catenin that interacts with HMR-1/cadherin (acts on cell adhesion) (Herman, 2002). SYS-1 β-catenin acts on a divergent canonical Wnt/β-catenin pathway, which controls asymmetric cell divisions of germ-line cells (Korswagen, Herman and Clevers, 2000; Kidd *et al.*, 2005).

Thus, unlike in vertebrates, where non-canonical Wnt signaling does not use the β-catenin protein, *C. elegans* has β-catenin functions distributed in four proteins: BAR-1 binds to POP-1, the TCF homolog of *C. elegans* and activates transcription of canonical Wnt signaling target genes (Lin et al., 1998; Korswagen et al., 2000; Herman, 2001); WRM-1 activates Nemo-like kinase to down-regulate POP-1 (Thorpe *et al.*, 1997; Rocheleau *et al.*, 1999); SYS-1 controls asymmetric cell divisions of germ-line cells (Sawa and Korswagen, 2013); and HMP-2 binds to HMR-1/cadherin but not to POP-1 acting on cell adhesion (Liu *et al.*, 2008; Sawa and Korswagen, 2013) (Figure 2A and 2B).

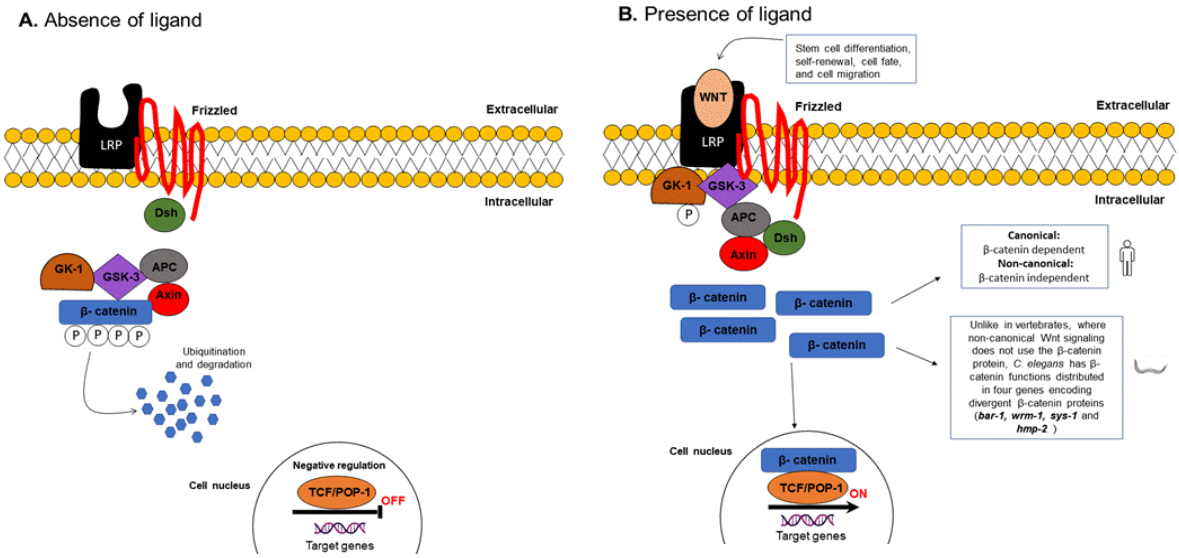

Figure 2. Wnt signaling pathways in the absence and presence of ligands in *C. elegans*. (A) Without signaling (absence of ligand), CK-1, GSK-3, Axin, and APC (negative regulators) phosphorylate β- catenin, which is ubiquitinated and degraded by the proteasome. (B) With signaling (presence of ligand), β-catenin stabilized in the cytoplasm is translocated to the nucleus, where it activates the expression of target genes. LRP: lipoprotein receptor; Dsh: Disheveled proteins; CK-1: casein kinase 1; GSK-3: Glycogen Synthase Kinase 3; APC: Adenomatous polyposis coli. Modified from Kobet *et al.*, 2014.

2.5 Notch signaling pathway

The Notch is a highly conserved signaling pathway that controls proliferation, differentiation, cell fate specification, and apoptosis, thus being critical for controllingdevelopment and homeostasis (Artavanis-Tsakonas, Rand and Lake, 1999). In mammals, four receptors of the Notch family have been described: NOTCH1, NOTCH2, NOTCH3, and NOTCH4. Most of its ligands (DSL: Delta/Serrate/LAG-2) are transmembrane proteins expressed in cells close to cells that express Notch

(Gridley, 2003; Kobet *et al.*, 2014). In the absence of ligands, transcription factors (CSL: CBF1, Hairless Suppressor, LAG-1) are associated with a complex that inhibits the expression of Notch target genes. When Notch interacts with a ligand, an ADAM metalloprotease family cleaves the outside of the Notch receptor. After this cleavage, γ-secretase cleaves the remaining part of the Notch receptor within the cell's inner membrane, causing the release of the Notch intracellular domain (NICD), which translocates to the nucleus. In the nucleus, NICD forms a complex with CSL transcription factors and mastermind-like protein (MAML-1) to activate target gene expression (Kirienko, Mani and Fay, 2010; Kobet *et al.*, 2014) (Figure 3). The role of Notch in stem cell regulation has been extensively studied in several model systems (Lee *et al.*, 2016; Enomoto, Takemoto and Igaki, 2021; Wei *et al.*, 2022). It has been known that exacerbated activation of Notch signaling has been detected in a variety of human cancers, including pancreatic câncer (Ristorcelli and Lombardo, 2010; Avila and Kissil, 2013), colon câncer (Miyamoto and Rosenberg, 2011), osteosarcoma (Galluzzo and Bocchetta, 2011) and breast cancer (Reedijk, 2012). In addition, Notch1 mutations in humans are observed in most cases of acute lymphoblastic leukemia (Ferrando, 2009; Kobet *et al.*, 2014). Thus, further investigations into this pathway are of interest, seeking a potential target for therapy against these types of cancer.

C. elegans has two Notch receptors (GLP-1 and LIN-12), which mediate cell-cell interaction during cell development (Greenwald, 2005). Mitotically dividing germ cells express the GLP-1/Notch receptor at all stages (Byrd *et al.*, 2014), and GLP-1/Notch signaling controls the maintenance of a pool of germ stem cells (GSCs) along the lifespan of the worm (Austin and Kimble, 1987; Kimble and Crittenden, 2007; Morgan, Lee and Kimble, 2010). When a GLP-1/Notch ligand is expressed in DTCs (Henderson *et al.*, 1994) and interacts with the GLP-1/Notch receptor, cleavage of the GLP-1/Notch receptor initiates, causing the release of NICD (Notch intracellular domain), which translocates to the nucleus and forms a tertiary complex with LAG-1/CSL (Figure 3). In the nucleus, the NICD forms a tertiary complex with the DNA binding protein LAG-1/CSL and the transcriptional coactivator LAG-3/SEL-8/Mastermind to activate the expression of the target genes: *fbf-2* (binding protein to RNA PUF) (Lamont *et al.*, 2004), *lip-1* (MAPK phosphatase) (Berset *et al.*, 2001; Lee *et al.*, 2006), *lst-1* (activates G protein)

(Kershner *et al.*, 2014), and *sygl-1* (required for germline stem cell maintenance) (Kershner *et al.*, 2014). The loss of GLP-1/Notch signaling in the germline causes a severe proliferation defect during the early meiotic phase, resulting in no maintenance of germline stem cells and sterility (Austin and Kimble, 1987). On the other hand, exacerbated activation of this signaling promotes proliferation of germline stem cells and their progenitor cells, as well as inhibits entry into meiosis, resulting in germline tumors and sterility (Berry, Westlund and Schedl, 1997), requiring efficient regulation of this pathway. Indirectly, other pathways can affect Notch signaling (Kirienko, Mani and Fay, 2010). One established target is FBF-2 and FBF-1, a member of the Pumilio and FBF (PUF) family, both expressed in the distal mitotic region of the germ line where GLP-1 is active (Lamont *et al.*, 2004). Although mutations in FBF-2 and FBF-1 are still unclear, germline proliferation stops after the L4 larval stage in *fbf-1/fbf-2* double mutants, indicating that these are necessary to maintain the cell-niche stem cells in adults (Crittenden *et al.*, 2002) promoting polyadenylation and increasing *gld-1* mRNA stability (Schmid, Küchler and Eckmann, 2009).

C. elegans has several Notch mutants, including *glp-1(q46)* or *glp-1(q175)* null mutants, *glp-1(bn18 or q224)* temperature-sensitive mutants (ts), with loss of function (lf), and temperature-sensitive *glp-1(ar202)* (ts) with gain-of-function mutant (gf) (Kobet *et al.*, 2014) (Table 1). When exposed to 20ºC or less, loss-of-function temperature-sensitive mutants for *glp-1 (glp-1(bn18)* and *glp-1(q224)*, produce sperm and oocytes and are fertile. On the other hand, at 25ºC, they have defective proliferation and early meiosis. However, a temperature-sensitive gain-of-function mutant for *glp-1(ar202)* with constitutively active GLP-1/Notch signaling promotes germ stem cell proliferation and inhibits entry into meiosis, resulting in germline tumors at 25°C (Pepper *et al.*, 2003). This phenotype (germ tumor) is rescued by the depletion of components of GLP-1/Notch signaling, including LAG-3/SEL-8/mastermind (Petcherski and Kimble, 2000). In *C. elegans*, the GLP-1/Notch signaling pathway and its main components are highly similar to humans, making it a potentialtool for investigating cancer therapies.

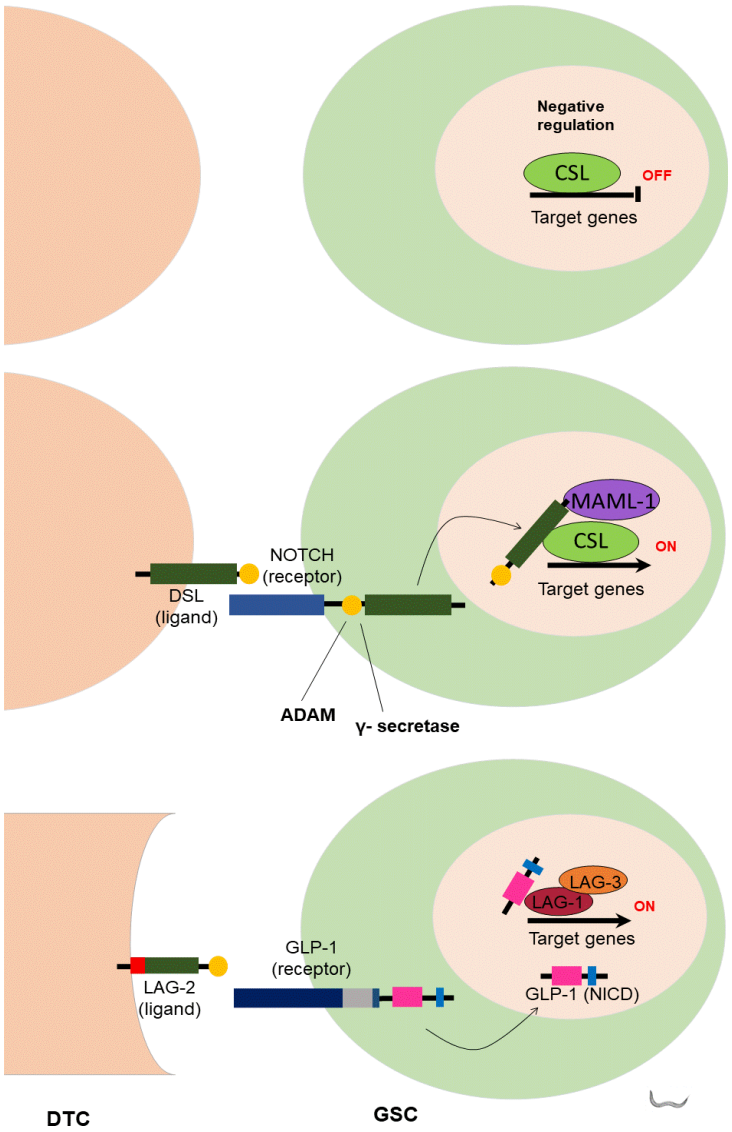

Figure 3. Notch signaling pathways in the absence and presence of ligands. (A) Without signaling, negative regulators inhibit the expression of Notch target genes. (B) With signaling, cleaved NICD is transported from the membrane to the core. In the nucleus, where they form a complex with CSL and co-activator MAML-1 to activate gene expression. (C) GLP-1/Notch signaling pathway in *C. elegans.* DTC expresses GLP-1/Notch ligands and employs GLP-1/Notch signaling to promote continuous mitotic division. MAML-1: mastermind-like protein; CSL: transcription factors CBF1/Hairless Suppressor/LAG-1; DSL: ligands Delta/Serrate/LAG-2; DTC: distal tip cell; GSC: germ stem cells; NICD: notch intracellular domain. Modified from Kobet *et al.*, 2014.

2.6 Tumor suppressors p53 and BRCA-1

In humans, changes in tumor suppressors p53 and BRCA-1 can be observed at all stages of neoplasm formation, thus these genes become molecular targets for cancer studies (Schumacher *et al.*, 2001; Raimundo *et al.*, 2020; Russi *et al.*, 2022). The p53 and BRCA-1 profile assessment can help identify patients with pre-malignant conditions.

The p53 or TP53 acts as a transcription factor inducing proteins that regulate the cell cycle by pausing the cell cycle, repairing errors found in the genome, or destroying the cell before it can cause damage to the organism (Fitzgerald L, 2016; Williams and Schumacher, 2016). p53 is the most commonly mutated tumor suppressor gene in human cancers (Rivlin *et al.*, 2011). Most of these mutations occur at its DNA binding site, which prevents it from completing its work as a transcription factor. One of the proteins initiated by p53 is p21, a cyclin-dependent kinase (CDK) inhibitor. CDKs are responsible for allowing a cell to progress through the cell cycle. If the CDK is blocked, the cell cycle stops and prevents further proliferation or replication of damaged DNA (Fitzgerald L, 2016). Other proteins governed by p53 are Siah-1, responsible for the degradation of β-catenin, a protein that induces the expression of cyclin D1 (another critical component for cell cycle progression). p53 also determines when a cell is beyond repair and initiates apoptosis by acting as a transcription factor. p53 induces the expression of a variety of intrinsic pro-apoptotic proteins, such as Bax and PUMA (Fitzgerald L, 2016). These proteins open cytochrome C channels in mitochondria. Once cytochrome C is in the cytoplasm, it activates caspases that cleave death substrates. The degradation of these death substrates initiates a cascade of cellular changes associated with apoptosis. Another intrinsic factor influenced by p53 is the FOXO3 transcription factor. p53 also induces the expression of insulin-like growth factor-binding protein (IGFBP-3), which binds to insulin-like growth factor (IGF) and prevents it from binding to its receptor, IGF-R1 (Oren, 2003; Harris and Levine, 2005; Fitzgerald L, 2016). The binding inhibition blocks survival signals to the cell, which favors apoptosis. p53 governs not only intrinsic but also extrinsic apoptotic factors, inducing the expression of the gene encoding the FAS receptor (Fitzgerald L, 2016). When FAS ligand binds to the receptor on the cell surface, a cascade of caspases is activated to cleave dead substrates. Thus, an alteration in the p53 gene can result in severe consequences. Under normal conditions, a cell that has accumulated dangerous mutations will self-destruct before it has the opportunity to transform. However, individuals with a mutation in this critical tumor suppressor gene accumulate mutations in their cells as repair is difficult. The p53 mutation in humans results in Li- Fraumeni syndrome, which is a disorder that predisposes individuals to breast cancer,

osteosarcoma, leukemia, brain tumor, and other cancers (Bell *et al.*, 1999; Gasco, Shami and Crook, 2002).

In *C. elegans, cep-1* was identified as the mammalian p53 homolog. Studies show that *cep-1* is required for DNA damage-induced apoptosis in the *C. elegans* germline (Schumacher *et al.*, 2001; Kirienko, Mani and Fay, 2010). The DNA-binding domain of CEP-1 is related to p53 members of vertebrates and has the most frequently mutated conserved residues in human tumors. CEP-1 acts as a transcription factor and can activate a transcriptional reporter containing human p53 binding sites. A cooperative role for the CEP-1 protein and the meiotic protein HIM-5 in maintaining genome stability in the *C. elegans* germline has been identified (Mateo *et al.*, 2016). *cep-1* and *him-5* also suppress inappropriate activation of the nonhomologous end-junction (NHEJ) pathway, demonstrating the role of the p53 family in ensuring meiosis fidelity and establishing CEP-1 as a critical determinant of repair pathway choice (Mateo *et al.*, 2016).

Several distinct apoptosis pathways have been characterized in the *C. elegans* germline (Salinas, Maldonado and Navarro, 2006). The physiological pathway eliminates excess germ cells during oogenesis to maintain gonad homeostasis and is activated by unknown mechanisms. DNA damage-induced germ cell apoptosis occurs in response to genotoxic agents and involves *egl-1* (BH-3 domain protein), *ced-9* (BCL-2 homolog), *ced -4* (Apaf-1 homolog), *ced-3* (caspase), *ced-13* (caspase), and the DNA damage response protein p53 (Schumacher *et al.*, 2001, 2005; Salinas, Maldonado and Navarro, 2006). Germ cell apoptosis can also be induced in response to infection by pathogens through an EGL-1-dependent pathway. However, oxidative, osmotic stress, heat shock, and starvation induce germ cell apoptosis through a p53 and EGL-1-independent pathway. In this case, MAPK kinases MEK-1 and SEK-1, and ABL-1 (p53 antagonist protein) are essential for stress-induced germ cell apoptosis (Salinas, Maldonado and Navarro, 2006). Thus, in *C. elegans*, responses to various stresses that do not involve genotoxicity include increased germ cell apoptosis through the physiological pathway. These results indicate that p53-mediated transcriptional regulation is part of a pathway that mediates DNA damage-induced apoptosis and makes *C. elegans* a genetically tractable model organism to study the p53 apoptotic

pathway.

Type 1 breast cancer susceptibility protein (BRCA-1) has been identified as the first major tumor suppressor gene associated with hereditary breast câncer (Petrucelli, Daly and Pal, 2022). Despite the wide range of activities already attributed to BRCA-1, its tumor suppressor function is mainly due to its ability to maintain genomic integrity, through which impaired BRCA-1 has been implicated in carcinogenic events from onset to progression (Futreal *et al.*, 1994). Its key role in maintaining genomic integrity occurs through the regulation of diverse cellular processes, including DNA damage repair (DDR) through homologous recombination (HR), non-homologous end-joining or single-stranded hybridization, cell cycle progression, maintenance of telomeres, chromatin remodeling, apoptosis and tumor suppression (Lancaster *et al.*, 1996). However, the effect of BRCA-1 on HR seems to be the most relevant mechanism in protecting genome integrity since mutant BRCA-1 tumors are usually defective in this DNA repair mechanism (Lancaster *et al.*, 1996; Scully *et al.*, 1997). In addition to BRCA-1, BRCA-2 is also required for cell homeostasis. With a mutated BRCA-1 or 2, the cell cannot repair, which leads to an accumulation of mutations that lead to proliferation and metastasis. Much is still unknown about BRCA-1 and 2, especially as loss of function primarily results in breast and ovarian cancer. BRCA-1 mutation carriers have a lifetime risk of breast cancer greater than 80% (Petrucelli, Daly and Pal, 2022). In addition to breast cancer, women with BRCA-1 mutations have an increased risk of ovarian cancer and, to a much lesser extent, men have an increased risk of prostate câncer (Fitzgerald L, 2016). Carriers of the BRCA-2 mutation are at increased risk of breast cancer in men and women, and cancer of the ovary, prostate, pancreas, gallbladder, bile duct and stomach, and melanoma. BRCA-1 also mediates cell cycle arrest as cells lacking BRCA-1 have alterations in the G1/S response to DNA damage (Gasco, Shami and Crook, 2002; Fitzgerald L, 2016).

In *C. elegans* Ce-*brc-1* (BRCA-1 ortholog) and Ce-*brd-1* (BARD-1 ortholog) work together in a common DNA repair pathway, similar to the role of BRCA-1 and BARD-1 in cells of mammals (Boulton *et al.*, 2004). Worms depleted of Ce-*brc-1* and Ce-*brd-1* have given rise to a Him phenotype (increased levels of X-chromosome nondisjunction) that can be caused by a defect during meiotic prophase or by a pre- altered meiotic. Under

normal growth conditions, an increase in germ cell death following depletion of Ce-*brc-1* or Ce-*brd-1* was also observed, suggesting that unrepaired DNA damage leads to cell death following depletion of Ce-*brc-1* or Ce-*brd-1*. In addition, treatment of Ce-*brc-1* or Ce-*brd-1* depleted worms with γ-irradiation results in increased germ cell death, radiation sensitivity, and chromosome fragmentation (Boulton *et al.*, 2004). Together, these phenotypes strongly suggest that Ce-BRC-1 and Ce-BRD-1 are required for DNA repair in *C. elegans*.

Table 1. Summary of oncogenic signaling pathways, genes involved, phenotypes, human homologs, and candidate drugs for cancer therapy in *C. elegans* studies.

Signaling pathway	Worm genes	Mammalian homolog	Phenotype	Related human cancer	Tested drugs	Ref.
Signaling pathway	**Worm genes**	**Mammalian homolog**	**Phenotype**	**Related human cancer**	**Tested drugs**	**Ref.**
RAS/RAF/ MEK/ERK	*mpk-1(ga117)*	MAPK1	Pachytene arrest and sterile	Endocrine gland cancer	AG1478 (EGFR inhibitor)	(Silva *et al.*, 2009; Guo *et al.*, 2020)
	*mpk-1(ga111)*ts	MAPK1	Multi-layered small oocytes and Multivulva (Muv)	Gastrointestinal system cancer	U0126 (MEK inhibitor)	
	let-60(n1046)	HRAS	Mog sterile at 20°C	Melanoma	Gliotoxin Itraconazole Disulfiram	
	puf-8(q725) lip-1(zh15)	PUM1	Germline tumors at 25°C	Non-small cell lung cancer	Etodolac Ouabain Mnumicin Gefitinib	
	lin-45	DUSP6	Sterility	Colorectal câncer		
	mek-2	RAF		Cutaneous melanoma		
	ksr-2	MEK		Acute myeloid leucemia		
		KSR2		Juvenile myelomonocytic leukemia		

ESPL1	*sep-1*	espl-1	Embryonic lethality	Several		
			Formatin of an impermeable eggshell		-	(Melesse *et al.*, 2018)
			Chromosomal disjunctin			
			Defective action on cell Division			
FOS	*fos-1*	c-fos	Anchor cell invasion into the vulval epithelium	Bone câncer	*c-Fos* inhibitor (T-5254) in conjunction with Eribulin in breast cancer	(Tanaka *et al.*, 2020)
				Skin câncer		
				Brain câncer		
				Pancreatic câncer		
				Ovary câncer		
				Sarcomas		
				NSCLCs		
				Breast câncer		
Wnt	*pop-1(q645)*	TAF	Total loss of DTC and sterility	Thyroid carcinoma	Itraconazole	(Herman, 2002; Eisenmann, 2005; Kobet *et al.*, 2014; Medina, Ponce and Cruz, 2021)
	sys-1(q544)	β-catenin		Leukemia	Disulfiram	
	lin 17(n671)	FZD4	Total loss of DTC and sterility	Breast cancer	Etodolac	
	hs::sys-1	β-catenin		Ovarian cancer	Ouabain	
	ceh-22(q632)	Nkx2.5	Loss of DTC (<10%) and partial sterility			
	hs::ceh-22	Nkx2.5				
			Loss of DTC (~40%) and partial sterility			
			Extra DTC and fertility			
			Extra DTC and fertility			

Notch	*glp-1(q46)*			Pancreatic cancer	Itraconazole	(Kirienko, Mani and Fay, 2010; Kobet *et al.*, 2014; Medina, Ponce and Cruz, 2021)
	*glp-1(bn18)*ts	NOTCH 1	Sterility	Colon cancer	Disulfiram	
	*glp-1(q224)*ts	NOTCH 2		Glioblastoma	Etodolac	
	*glp-1(ar202)*ts	NOTCH 3		Breast cancer	Ouabain	
				Leukemia		
p53 and BRCA-1	*cep-1*	p53	Loss of function makes tumor formation susceptible	Breast cancer	-	(Boulton *et al.*, 2004; Kirienko, Mani and Fay, 2010; Mateo *et al.*, 2016)
	brc-1	BRCA-1		Osteosarcoma		
	brc-2	BRCA-2		Leukemia		
	brd-1	BARD-1		Brain tumors		
				Prostate cancer		
RAS/RAF/ MEK/ERK	*mpk-1(ga117)*	MAPK1	Pachytene arrest and sterile	Endocrine gland cancer	AG1478 (EGFR inhibitor)	(Silva *et al.*, 2009; Guo *et al.*, 2020)
	*mpk-1(ga111)*ts	MAPK1	Multi-layered small oocytes and Multivulva (Muv)	Gastrointestinal system cancer	U0126 (MEK inhibitor)	
	let-60(n1046)	HRAS	Mog sterile at 20°C	Melanoma	Gliotoxin Itraconazole Disulfiram	
	puf-8(q725) lip-1(zh15)	PUM1	Germline tumors at 25°C	Non-small cell lung cancer	Etodolac Ouabain Mnumicin Gefitinib	
	lin-45	DUSP6	Sterility	Colorectal cancer		
	mek-2	RAF		Cutaneous melanoma		
	ksr-2	MEK		Acute myeloid leukemia		

		KSR2		Juvenile myelomonocytic leukemia		
ESPL1	*sep-1*	espl-1	Embryonic lethality	Several		
			Formatin of an impermeable eggshell		-	(Melesse *et al.*, 2018)
			Chromosomal disjunctin			
			Defective action on cell division			
FOS	*fos-1*	c-fos	Anchor cell invasion into the vulval epithelium	Bone cancer	*c-Fos* inhibitor (T-5254) in conjunction with Eribulin in breast cancer	(Tanaka *et al.*, 2020)
				Skin cancer		
				Brain cancer		
				Pancreatic cancer		
				Ovary cancer		
				Sarcomas		
				NSCLCs		
				Breast cancer		
Wnt	*pop-1(q645)*	TAF	Total loss of DTC and sterility	Thyroid carcinoma	Itraconazole	(Herman, 2002; Eisenmann, 2005; Kobet *et al.*, 2014; Medina,
	sys-1(q544)	β-catenin		Leukemia	Disulfiram	
	lin 17(n671)	FZD4	Total loss of DTC and sterility	Breast cancer	Etodolac	
	hs::sys-1	β-catenin		Ovarian cancer	Ouabain	

	ceh-22(q632)	Nkx2.5	Loss of DTC (<10%) and partial sterility			Ponce and Cruz, 2021)
	hs::ceh-22	Nkx2.5				
			Loss of DTC (~40%) and partial sterility			
			Extra DTC and fertility			
			Extra DTC and fertility			
Notch	*glp-1(q46)*			Pancreatic cancer	Itraconazole	(Kirienko, Mani and Fay, 2010; Kobet *et al.*, 2014; Medina, Ponce and Cruz, 2021)
	*glp-1(bn18)*ts	NOTCH 1	Sterility	Colon cancer	Disulfiram	
	*glp-1(q224)*ts	NOTCH 2		Glioblastoma	Etodolac	
	*glp-1(ar202)*ts	NOTCH 3		Breast cancer	Ouabain	
				Leukemia		
p53 and BRCA-1	*cep-1*	p53	Loss of function makes tumor formation susceptible	Breast cancer	-	(Boulton *et al.*, 2004; Kirienko, Mani and Fay, 2010; Mateo *et al.*, 2016)
	brc-1	BRCA-1		Osteosarcoma		
	brc-2	BRCA-2		Leukemia		
	brd-1	BARD-1		Brain tumors		
				Prostate cancer		

3. *C. elegans* as a tool for early cancer detection

In the last decades, the methods to diagnose cancer evolved significantly, which propitiated the early detection and treatment of this disease and, consequently, increased the life expectancy of the subjects affected (Falzone, Salomone and Libra, 2018). However, the costs of these tests used in the initial diagnosis of some neoplastic

processes are high for the health systems. In the EUA, it is estimated that people with cancer present approximately 61% more medical expenditures than healthy people, being that low-income people have difficulties obtaining an early diagnosis of cancer and, therefore, higher death rates (Brill, 2020). Due to this problem, it is necessary to optimize inexpensive tools for early cancer screening to minimize costs in suspected cases(Round T, 2021).

In this perspective, *C. elegans* has been studied as a tool for the initial screening of cancer diagnosis in the assay known as *Nematode Scent Detection Test* (NSDT) or N-NOSE. In this test, the worms are placed in NGM (Nematode Growth Medium) plates, and on each border of the plate is found the presence of an attractive (cancer cells) or control group. As a result, the nematodes migrated to samples with cancer. In addition to being cheap, this method provides fast results. This method is based only on the chemosensory ability of nematodes through their olfactory neurons (AWC and AWA), in which the transmembrane guanylyl cyclase (ODR-1) plays the role of perceiving different odors, which may or may not attract animals (Hirotsu *et al.*, 2015; Shidara, Hotta and Oka, 2017). This chemotaxis behavior is well known in *C. elegans,* and is important for the worms to know which bacteria they can feed or not due to the presence of different volatile odorants released for these microorganisms (Worthy *et al.*, 2018).

The NSDT was used with urine samples of patients with or without cancer, in a medium used to maintain human cancer (colorectal, breast, and gastric cancer) and normal fibroblast cell lines or with human cancer tissue. In this test, the worms were submitted to chemotaxis assays with these different samples diluted and demonstrated attraction to the secretions of individuals with cancer in all cases. Inaddition, the test showed a high sensibility (95,8%) and specificity (95,0%) of NSDT for various types of early-stage cancer (Hirotsu *et al.*, 2015).

Other studies with this same method validated the NSTD efficacy. Ishii et al.(2019) used *C. elegans* to detect pancreatic tumors (in mice) using urine. The worms demonstrated a perception of odor's presence in the samples of animals with cancer (Ueda *et al.*, 2019). In diluted urine (1:1000) obtained from individuals with prostate

cancer, the worms presented 81% precision in detecting this type of cancer. The VOCs (volatile organic compounds) 2-octonone and Pentanal, biomarkers reported in the urine of men with this type of cancer, are known chemoattractants for worms (Thompson *et al.*, 2021).

An improvement in this method (N-NOSE), with two dilutions, 10-fold, and 100-fold, increased detection sensitivity to 87.5% in urine samples of patients with cancer (esophageal, gastric, colorectal, gallbladder, cholangiocarcinoma, breast, malignant lymphoma, and acute myeloid leukemia) in different stages. These results are better than those using some blood biomarkers to detect these types of cancer mentioned (Inaba *et al.*, 2021). When the chemotaxis with urine samples was correlated with calcium imaging of AWC neurons, the sensibility of breast cancer detection increased to 97.22%. However, a limitation in women's hormonal cycle was found, particularly in the follicular and periovulatory phases (Lanza *et al.*, 2021). In humans, other cancer types, such as gastrointestinal and pancreatic, were also detected in urine samples, with high sensitivity and specificity (KUSUMOTO *et al.*, 2020; Kobayashi *et al.*, 2021).

In some cancer cases, chemotherapy is not enough to reverse the abnormal growth of tumor cells. Depending on the stage in which this disease is diagnosed, the surgical removal of the tumor is necessary. After this process, the monitoring of patients is necessary in order to prevent the recurrence of tumors. These exams may have a high cost and be invasive (Tohme, Simmons and Tsung, 2017). In this situation, *C. elegans* was also tested for monitoring recovery and regression of metastasis processes in operated patients. The study demonstrated that the worms are less attracted to postoperative urine samples than samples collected before tumor removal (Kusumoto *et al.*, 2019).

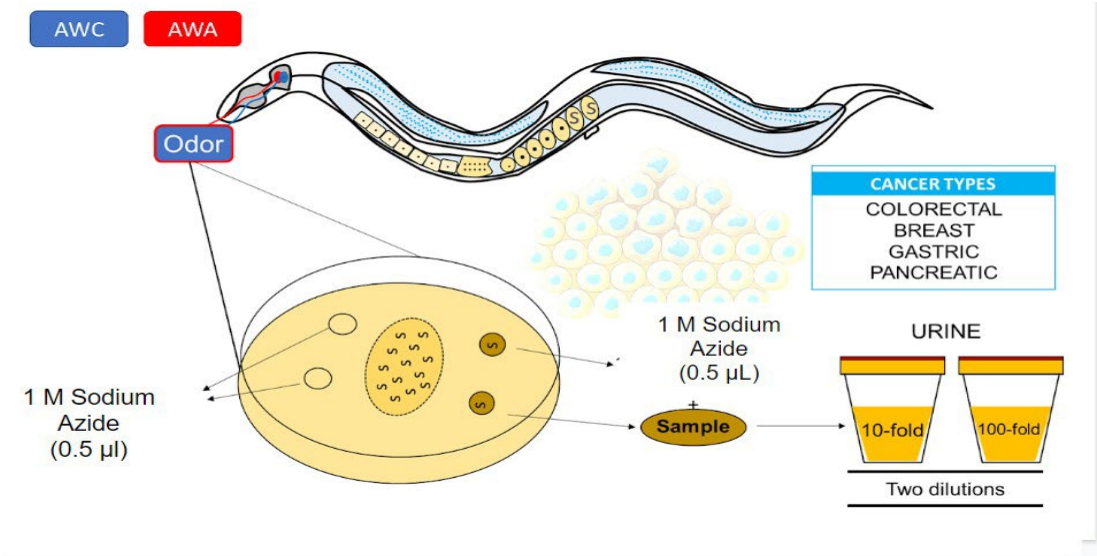

Figure 4. NSDT or N-NOSE method in urine as a sample to cancer detection using *C. elegans.* Modified from Thompson *et al.*, 2021 and Inaba *et al.*, 2021.

4. *C. elegans as a model for drug Discovery in cancer*

Drug discovery is a crucial part of developing new cancer treatments. Usually, in vitro models are used as cancer models because of the highly controlled conditions, homogeneity, discovery of molecular mechanisms, reproducibility, and the ability to perform screenings of large quantities of chemicals simultaneously (Cekanova and Rathore, 2014). This process has been aided by the advance of bioinformatics which allows performing screenings of thousands or even millions of chemicals using computational software, helping to reduce experimental costs, accelerate drug target identification, and drug candidate screening and refinement (Xia, 2017). Animal models are still necessary since the biological responses can differ from in vitro to *in vivo*. These animal models include mainly rodents and zebrafish (Cekanova and Rathore, 2014) because of the high genetic homology with humans. However, preclinical studies using these animals are costly, time-consuming, and can only be performed with a limited number of compounds. Therefore, C. elegans has been considered an excellent alternative as an *in vivo* model.

The use of C. elegans in cancer research has increased in the last years (Figure 5) and within its interest as an *in vivo* model for screening anticancer drug candidates. The capability of genetic manipulation in C. elegans is one of the main strengths as a

model to study cancer for the discovery of mechanisms or new pathways involved and for the discovery of new drugs with the potential to be used in cancer treatment (Kobet et al., 2014). As mentioned before, mutations in the signaling pathways observed in cancer in humans, such as in Wnt, Notch, and Ras-ERK signaling cascades (Reiner et al., 2008), lead to defective signaling pathways also in *C. elegans*, however, instead of forming cancer, the *C. elegans* mutant strains become sterile, infertile, and form multivulva (Muv). Thus, drugs that can reverse these mutant phenotypes in *C. elegans* may also potentially reverse cancer in humans (Kobet et al., 2014; Medina, Ponce and Cruz, 2021). Another advantage of using *C. elegans* for drug discovery is the possibility of developing high-throughput and low-cost *in vivo* screening systems to test hundreds or even thousands of molecules at the same time (Bae et al., 2012), making this model an excellent alternative for a screening system, with the practicality of in vitro screening systems and the biological relevance of animal models (Artal-Sanz, de Jong and Tavernarakis, 2006).

Screening of candidate drugs with *C. elegans* can be performed through drug repurposing, a faster and cost-effective way of finding new drugs for certain diseases such as cancer. Compared to the conventional way of drug discovery, concerns about toxicity can be avoided because the selected drugs already have well-documented safety profiles (Pushpakom et al., 2019).

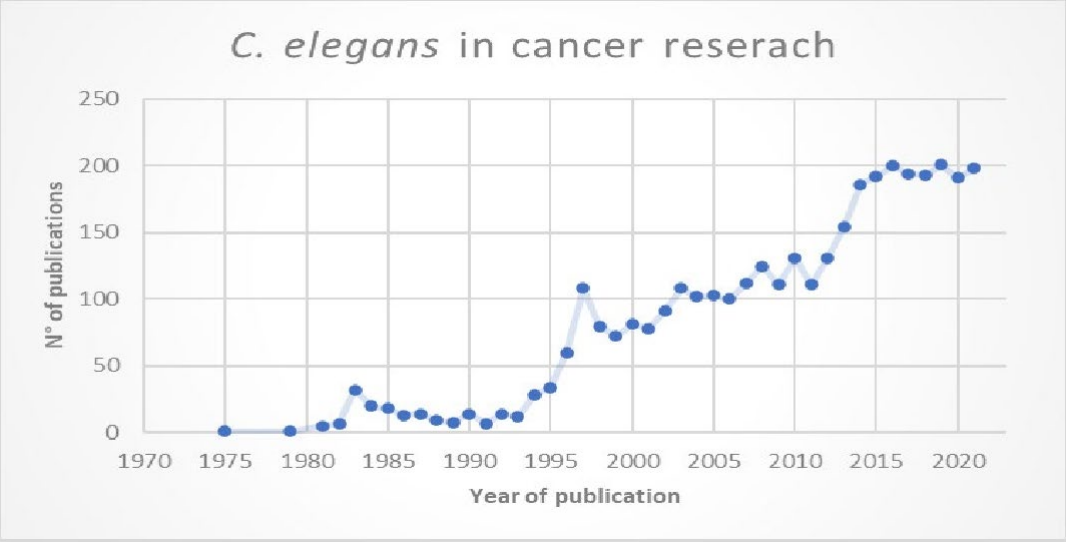

Figure 5. Publications of *C. elegans* in cancer research. Data extracted from Pubmed (obtained on 04/01/2022).

4.1 Compounds studied as potential anticancer drugs

Here we present a diversity of compounds that have already been studied as potential anticancer drugs using *C. elegans* as an *in vivo* model.

4.1.1 Chemical compounds

EGFR is overexpressed or aberrantly activated in various types of human cancer, such as breast, ovarian, and non-small-cell lung carcinoma (Hynes and Lane, 2005), providing an attractive target for cancer drug development. In addition, components of the EGFR pathway are highly conserved between humans and *C. elegans* (Chang and Sternberg, 1999). Using constructed transgenic *C. elegans* containing several different EGFR constructs, Bae et al. (2012) conducted a pilot screen of 8,960 chemicals, isolating an EGFR inhibitor (AG1478) and a MEK inhibitor (U0126) as potential inhibitors of EGFR-mediated biological function. In this concern, they developed a protocol for large-scale high-throughput screening of EGFR inhibitors. This study suggests that this *C. elegans*-based system can be used efficiently to screen for new anticancer drugs on a large scale (Bae et al., 2012).

4.1.2 Natural compounds

Liu et al. tested the decoction of traditional antioxidant medicine called Kushui rose (R. Setat x R. Rugosa) (KR) as an antitumor drug candidate. This study uses a *C. elegans* mutant (let-60(gf)) to determine whether KR can suppress an over-activated Ras/MAPK pathway. In *C. elegans*, let-60 is a homologous gene to ras in mammals, and Ras/MAPK signaling pathway determines the development of the worm vulva (Mattiasson, 2004).The over-activated Ras/MAPK pathway produces an abnormal multivulva (Muv) phenotype, which can be reversed by antitumor drug candidates such as KR. The results showed that KR can inhibit over-activated Ras *in vivo*, and KRD significantly suppresses the over-activated Ras/MAPK pathway by regulating oxidative stress-related proteins, such as forkhead transcription factor (DAF-16), glutathione S-transferase-4 (GST-4), superoxide dismutases (SODs) and heat shock protein-16.2 (HSP-16.2). This evidence that KR can serve as a potential drug candidate for combating over-activated Ras-related cancer (Liu et al., 2018).

Another natural product tested as a potential drug candidate for combating over-activated Ras-related cancer is Shengmai formula (SM), a traditional Chinese medicine formula that is composed of Radix Ginseng (Panax ginseng), Radix Ophiopogonis (Ophiopogon japonicus, and Fructus Schisandrae (Schisandra chinensis) (Wu et al., 2011). This ancestral formula has been used due to its strong antioxidant activity. Here, they also used a *C. elegans* mutant (let-60(gf)) to investigate whether SM formula can suppress over-activated Ras/MAPK pathway. The results evidenced that SM treatment opened mitochondrial permeability transition pore by regulating cyclophilin D and then triggered oxidative stress and related signaling pathway activation, including p53, JNK, and p38/MAPK pathways. Interestingly, SM acted as a pro-oxidant, induced mitochondrial pathway of apoptosis, and inhibited the tumor-like symptom of the multivulva phenotype of let-60(gf) mutants. Taken all together, SM appears to be a promising drug candidate against over-activated Ras-related cancer (Liu et al., 2016).

4.1.3 Using drug repurposing

The anticancer potential of itraconazole, disulfiram, etodolac and, ouabain was assessed by Medina et al.(2021), who used phenotypic assays of *C. elegans* mutant strains such us Wnt (JK3476), Notch (JK1107 and BS3164), and Ras-ERK (SD939 and MT2124) that result in phenotypes of sterility, infertility, and multivulva formation. As a result, the ability of these drugs to affect the Wnt, Notch, and Ras-ERK signaling pathways were shown since the drugs were able to rescue sterility, infertility, and Muv formation significantly. Both ouabain and etodolac significantly reduced the sterile and infertile phenotypes of JK3476, JK1107, BS3164, and SD939 strain, and itraconazole and etodolac significantly reduced multivulva formation. This work generally suggests that the four candidate drugs have anticancer potential *in vivo* (Medina, Ponce and Cruz, 2021).

4.2 Toxicological screening

C. elegans has also been studied to assess anticancer therapy's toxicity or potential side effects. For instance, neurotoxicity is a common side effect of the

treatment with cisplatin. It was demonstrated that cisplatin can cause the reduction of pumping frequency at concentrations where basal and touch-provoked movement were not yet affected. Measuring pharyngeal activity by the electrophysiological recording of neurotransmission in the pharynx confirmed that cisplatin is neurotoxic in *C. elegans*. The data support the hypothesis that monitoring the pharyngeal activity of *C. elegans* is a useful surrogate marker of cisplatin-induced neurotoxicity (Wellenberg et al., 2021). In addition, a dose and time-dependent cisplatin uptake was corroborated quantitatively by a total reflection X-ray fluorescence spectroscopy (TXRF) method, and the elemental mapping showed that cisplatin was predominantly located in the area of the intestine and in the head of the worms.

These results open the possibility of correlating toxicological alterations with the amount of drug and might be helpful for a better understanding of Cisplatin pharmacokinetics and dose-efficiency studies of Cisplatin (Crone et al., 2015).

5. Conclusion and perspectives

Cancer is a complex condition involving many genes conserved in *C. elegans* due to its high homology to humans. Such genes are essential for pathways involved in regulating the cell cycle and differentiation, apoptosis, and development of some tissues. When dysregulated due to mutations, phenotypes are observable in the germline formation or processes involving the worm's reproductive system, such as oocyte and sperm formation, impairment in the germline cells, or vulva formation.

Some *C. elegans* transgenic strains with mutations in genes involved in cancer in humans have been successfully used to screen new synthetic and natural compounds with anticancer potential. The effectiveness of these agents is proven mainly due to improvement in the phenotypic parameters analyzed, antioxidant potential, and activation of pathways involved in the apoptotic process. In addition, the safety of some anticancer candidates has also been investigated in *C. elegans*, such as cisplatin neurotoxic effects.

Besides that, this invertebrate can be used in cancer research to diagnose these diseases using human samples like urine or blood, with a high sensibility to different

types of cancer like gastrointestinal, pancreatic, prostate colorectal, malignant lymphoma, and acute myeloid leukemia, for example. This is possible due to the worm's chemosensory ability, which is a particular behavior that has been explored to this objective in cancer detection.

With advances in therapy research and the understanding of cancer pathways in *C. elegans,* this model has demonstrated to be an excellent tool as an *in vivo* model. However, there are few studies in this area with these nematodes up to this date. The studies are increasing and will advance, particularly with the development of new and more powerful strains using CRISPR/Cas9 technology. That will allow the initial screenings of new candidate drugs for cancer therapy and their side effects. The use of the nematode in the initial screening for the diagnosis of patients with neoplastic processes will also make it possible to minimize the execution of invasive tests in patients.

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Abbreviations

TBBPA - Tetrabromobisphenol A
GO - Graphene Oxide
MPs - Microplastics
PS-MPs - Polystyrene microplastic
ACh - Acetylcholine
Glu - Glutamate
GABA - γ-amino butyric acid
DA - Dopamine
5-HT - Serotonine
GFP - Green fluorescent protein
YFP - Yellow fluorescent protein
mCherry - mCherry fluorescent protein
AD - Alzheimer's disease
PD - Parkinson's disease
QP - Quinalphos
MCP -Monocrotophos
ATP - Adenosine triphosphate
CMs - Carbamate
OPs - Organophosphorus
CAR - Carbofuran
MET - Methomyl
CPF - Chlorpyrifos
TAP - Triazophos
LDH - Lactate dehydrogenase
ROS - Reactive oxygen species
SOD - Superoxide dismutase
CAT - Catalase
Aβ42 - Amyloid beta peptide
HSP - Heat shock protein

Chapter 3

Caenorhabditis elegans Immune System

Victória Regina da Silva
Matheus Chimelo Bianchini
Daniela Teixeira Rodrigues
Ana Maria Vieira da Silva
Rosana Pereira Nobre de Lima
Amanda Onduras de Andrade
Tábata Martins de Lima
Priscila Gubert

Abstract

Caenorhabditis elegans, a free-living nematode used as an experimental model in research, is constantly exposed to environmental and dietary stressors. Due to this, its immune system, composed only of innate immunity, plays a fundamental role in its survival, aging, and adaptation to stressful stimuli. Several conserved MAPK pathways and neuronal responses through biogenic amines act together to regulate all these processes. The aim of this work was to review the main regulatory pathways ofthe innate immune response of *C. elegans*, emphasizing the central role of p38 MAPK.The pathways' disclosing is useful for understanding the role and control of innate immunity in different organisms, considering that *C. elegans* has high genetic homology with other animals, including humans.

1. Introduction

The immune system is one of the most primordial systems in an organism's body. However, only in the last century, with advances in technology, it has begun to be better understood. The immune system can be divided into innate immunity or adaptive immunity. Innate immunity is considered the most important type of immunity. This system is responsible for fast and universal responses. It is a nonspecific defense line conserved among diverse organisms during evolution (Deborah A. Kimbrell & Beutler, 2001). Some individuals rely exclusively on the innate immune system. In most vertebrate and invertebrate animals, it is composed of immune cells, pattern recognition receptors (PRRs), and molecular factors that act

together to destroy pathogens (Garcia-Sanchez et al., 2021).

Innate immunity can be categorized into two paths: afferent or efferent, andboth have cellular and a humoral component. These categories are important, especially when scientists must study each one. The afferent arm is related to how the immune system is able to sense a foreign body and which structures or vias are involved. To sense when the body is in 'danger' is essential so the immune system can generate an appropriate response, activating the efferent arm (Beutler, 2004). The idea that the immune system needs to be studied from an integrative context is becoming more important each day (Harding & Ewbank, 2021).

During evolution, *Caenorhabditis elegans* conserved only molecules-based immunity. Despite the lack of other defense mechanisms, their absence makes *C. elegans* an excellent model for investigating the role of molecular pathways in the immune system without interference. This chapter will focus on the central pathways involved in immunity with a particular focus on p38 MAPK.

2. Central pathways involved in *C. elegans* immunity

C. elegans lacks most of the immunological components found in vertebrates and preserves only molecular elements involved in the innate immune response during evolution. Due to this, some conserved pathways are essential to promote defense responses, such as Insulin/insulin-like growth factor signaling (IIS) and Mitogen-activated protein kinase (MAPK) signaling, a group of tyrosine/threonine protein kinases activated by several stimuli, including environmental stress and pathogens, which includes p38 MAPK, ERK and c-Jun N-terminal kinase (JNK) signaling pathways (Berman et al., 2001; Fabian et al., 2021).

The p38 MAPK is an ancient and conserved family of proteins related to host defense (Kim et al., 2002). In *C. elegans*, an important member of the p38 MAPK family is the orthologue PMK-1, described in association with many pathways, including innate immunity response, pathogen response, and longevity (Troemel et al., 2006). Indeed, PMK promoter is highly expressed in intestinal cells, which corroborates with its role in protecting worms from external stresses (Berman et al., 2001). External

stimuli induce signal transduction inside the cell through cell receptors, which may vary depending on the stimulus. For example, Toll/interleukin-1 receptor/resistance protein (TIR-1) acts upstream PMK-1 pathway during pathogen resistance, such as *Pseudomonas aeruginosa* (Liberati et al., 2004), where a MAPK kinase kinase (MAPKKK) phosphorylates and activates a MAPK kinase (MAPKK), which phosphorylates the threonine/tyrosine residues of PMK-1, that induces the activation of transcriptional factors, usually members of the basic region leucine zipper (bZIP) family. NSY-1 (MAPKKK) and SEK-1 (MAPKK) have been described as upstream activators in PMK-1 activation (Kim et al., 2002). Next, downstream signaling is followed by phosphorylation of Activating Transcription Factor 7 (ATF-7), an orthologue of the human protein ATF2 converted from an inhibitor to an activator of the immune response mediated by PMK-1.

ATF-7 acts like a global regulator for most genes associated with the immune response, promoting the transcription of genes such as C-type lectin domain (CTLD)-containing genes and lysozymes (Fletcher et al., 2019; Shivers et al., 2010). Other transcription factors may be mobilized by the PMK-1 cascade when faced with different stressors. During heat stress, PMK-1 can translocate to the nucleus and activate transcription factor skinhead-1 (SKN-1) or heat-shock factor 1 (HSF1), triggering the transcription of heat-shock proteins (HSPs, also known as chaperones) in response to heat-stress (Mertensk̈otter et al., 2013). SKN-1 activation mediated by PMK-1 was also described as an inducer of the transcription of *gcs-1*, a gene that encodes the phase II detoxification enzyme, γ-glutamine cysteine synthase heavy chain in response to oxidative stress caused by arsenite (Inoue et al., 2005). Furthermore, the *C. elegans* mutants *tol-1*, *sek-1,* and *pmk-1* infected by *Klebsiella pneumoniae* did not develop worm defense response, showing the central role of PMK- 1 cascade in pathogen defense (Kamaladevi & Balamurugan, 2015).

JNK, also known as a stress-activated protein kinase, is another MAPK signaling family composed of JNK (JNK-1) and JNK-like (KGB-1 e KGB-2) MAPKs (Marudhupandiyan & Balamurugan, 2017). As well as p38 MAPK, JNK owns a cascade formed by MAPKKK, MAPKK, and MAPK proteins. While JNK-1 modulates locomotion

through type-D GABAergic motor neurons, when activated by JKK-1(MAPKK), KGB-1, and KGB-2 are mainly related to environmental stress response and pathogen resistance (Hattori et al., 2013; Kawasaki et al., 1999; Marudhupandiyan & Balamurugan, 2017). KGB-1 phosphorylation cascade is composed of MLK-1 (MAPKKK), MEK-1 (MAPKK), and KGB-1 (MAPK), which phosphorylate transcription factors (Mizuno et al., 2004). During heavy metals-induced stress, KBG- 1 phosphorylates and blocks the dimerization of FOS-1, a transcription factor involved in the repression of stress response that increases sensibility to copper. Inhibition of FOS-1 mediated by the JNK cascade allows transcription of KREG-1, which may act by chelating the copper through polyhistidine stretches, avoiding cell injury (Hattoriet al., 2013). Meanwhile, KGB-2 is important during infection by *Shigella flexneri* to activate the defense responses. In a crosstalk with the ISS pathway, KGB-2 promotes the activation of transcription factor DAF-16 that upregulates the expression of the lysozyme *lys-7* (Marudhupandiyan & Balamurugan, 2017).

Another crosstalk between JNK-MAPK and ISS pathways occurs when worms are submitted to heat stress, where JKK-1 is activated after cells sense environmental stress, stimulating JNK-1 that phosphorylates and promotes nuclear translocation of DAF-16, which was previously inhibited by ISS pathway. Thenceforth, a stress response is developed (Wook et al., 2005). VHP mediates negative regulation of JNK-like pathways, a MAPK phosphatase (MKP) that controls fine-tuning of the stress response by dephosphorylating and inactivating KGB-1 (Mizuno et al., 2004). An overview of pathways involved in *C. elegans* immunological responses is shown in Figure 1.

Studies of *C. elegans* immunity pathways are critical because they enable us to understand the roles of innate immunity in many conserved processes. An example is the discovery that the JNK pathway is upregulated during hypercapnia and induces deleterious effects in epithelial cells when carbon dioxide increases due to a decrease in the Na+/K+-ATPase pump. This negative effect is conserved among species and leads to epithelial dysfunction in humans, reinforcing the value of *C. elegans* model under the comprehension of the pathogenesis mechanism (Vadász et al., 2012). On the

other hand, another application for studying pathways in *C. elegans* is to aid in developing new drugs, such as immunomodulators. The study of the action of some immunomodulatory substances in *C. elegans* showed great efficacy in the resistance to pathogens by upregulating the p38 MAPK signaling pathway, allowing to pre-select them for future studies applied to humans. These studies are highly relevant because resistance to antibacterial is a worrying reality and finding alternative ways to destroy pathogens is an excellent advance (Hummell et al., 2021). From that, it is possible to observe that investigating conserved innate immunity pathways from *C. elegans* has several applications, as will be detailed in the topics below.

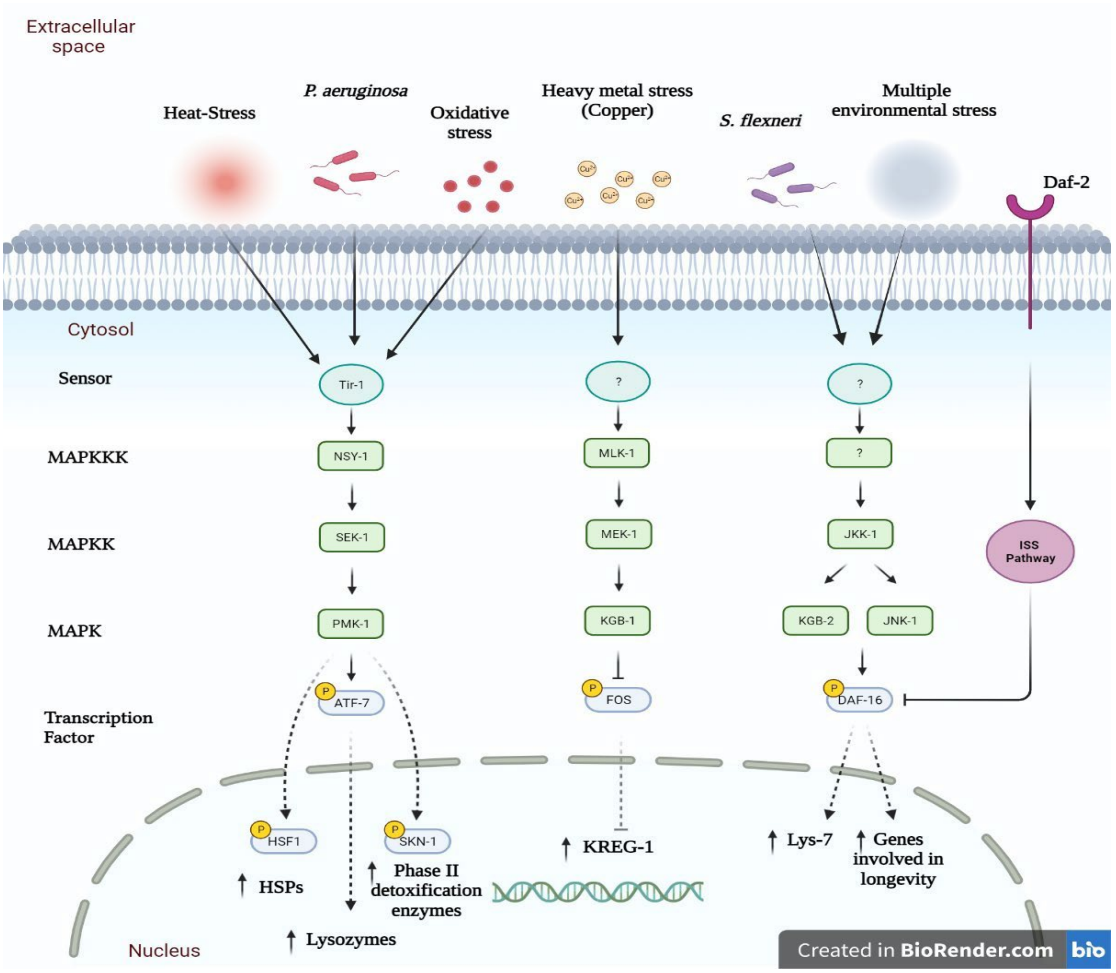

Figure 1. Main MAPKs' pathways and environmental stimuli involved in immune response activation. When *C. elegans* is exposed to stress and pathogens, several MAPKs' pathways are activated to produce a defensive response. PMK-1 is activated by heat stress, *P. aeruginosa*, and oxidative stress, in response phosphorylates the transcription factors HSF1, ATF-7, and SKN-1, respectively, inducing gene transcription. KGB-1 is activated during heavy metal stress and acts inhibiting FOS dimerization when phosphorylates it, which allows KREG-1 transcription. *S. flexneri* induces KGB-2 activation, which promotes Lys-7 transcription through DAF-16 phosphorylation. JNK-1 activates DAF-16 in response to multiple environmental stresses, promoting the transcription of genes involved in longevity. DAF-16 can be inhibited by the ISS pathway activation. ATF-7, Activating transcription factor 7; DAF-16,

abnormal dauer formation family member 16; DAF-2, abnormal dauer formation family member 2; FOS, B-Zip transcription factor homolog 1; HSF1, heat-shock factor 1; HSPs, heat-shock proteins; ISS, Insulin/insulin-like growth factor signaling; JKK-1, JNK Kinase; JNK-1, c-Jun N-terminal kinase 1; KGB-1, Kinase GLH-Binding 1; KGB-2, Kinase GLH-Binding 2; KREG-1, KGB-1 regulated gene 1; Lys-7, Lysozyme 7; MEK-1, Erk Kinase; MLK-1, Mixed lineage kinases member 1; NSY-1, Neuronal Symmetry family member; PMK-1, P38 Map Kinase family member 1; SKN-1, transcription factor skinhead-1; Tir-1, Toll/interleukin-1 resistance protein.

3. Biogenic amines and Immune System

The central nervous and immune systems quickly and precisely respond to the presence of pathogenic microbes in the environment. Whilst the immune system activates cellular defenses to recognize and eliminate pathogens, changes in neuronal signaling alter animal behavior to avoid these microbes.

The nervous system of *C. elegans* comprises only 302 neurons and 56 glial cells (White et al., 1986). Because of that, the worms are an attractive model to answer the questions of neuro-immune communications. Previous studies have highlighted the role of G protein-coupled receptors (GPCRs) in regulating *C. elegans* innate immunity (Aballay, 2013). A neural circuit involving *npr-1*, which encodes a GPCR homologous to mammalian neuropeptide Y receptors, imposes suppressive control of the innate immune response through the PMK-1/p38 MAPK pathway. The NPR-1 inhibits AQR, PQR, and URX sensory neurons. Genetic ablation of these neurons confers enhanced resistance to bacterial infections, implying that the neural circuit involving NPR-1-expressing neurons is responsible for immunosuppression during infection (Styer et al., 2008).

Biogenic amine neurotransmitters, such as dopamine, play significant roles in central and peripheral nervous systems, regulating multiple physiological functions, such as reward, memory, depression, and sleeping (Wise, 2004). These neurotransmitters are released from presynaptic vesicles and bind to the corresponding receptors, including GPCRs and ligand-gated ion channels, to induce intracellular second messenger cascades or changes in membrane potential. The dopamine signaling negatively regulates the innate immune response through a D1- like dopamine receptor, DOP-4, by down-regulating the p38/PMK-1 MAPK pathway. Furthermore, a putative neural circuit involving dopaminergic neurons CEP and DOP- 4-expressing neurons ASG has been

shown to control the immune response against pathogen infections (Figure 2). The ablation of CEP neurons conferred a similar enhanced resistance to infection, as observed in the *dop-4* mutant animals. In addition, exogenous dopamine suppressed the enhanced resistance to pathogen infection caused by CEP ablation. The identification of this neural circuit and the demonstration that chemical inhibition of dopamine signaling in the nervous system can control immune pathways at the cell non-autonomous level provides proof of concept for theuse of neural interventions to control infections and conditions that involve aberrantimmune functions (Cao & Aballay, 2016).

Indeed, dopamine levels also regulated expression of the genes in IGF-1/DAF-16 signaling (*age-1* and *daf-16*) and innate immune signaling pathways (*pmk-1* and *sek-1*) was significantly down-regulated in the mutant defective *cat-2* (tyrosine hydroxylase), and supplementation of dopamine to *cat-2* mutants restored the expression of these genes to the level in the wild type. Therefore, these studies indicate that dopaminergic signaling indeed has a role in regulating the IGF-1/DAF-16 and innate immune signaling pathways in *C. elegans* (Liu et al., 2020).

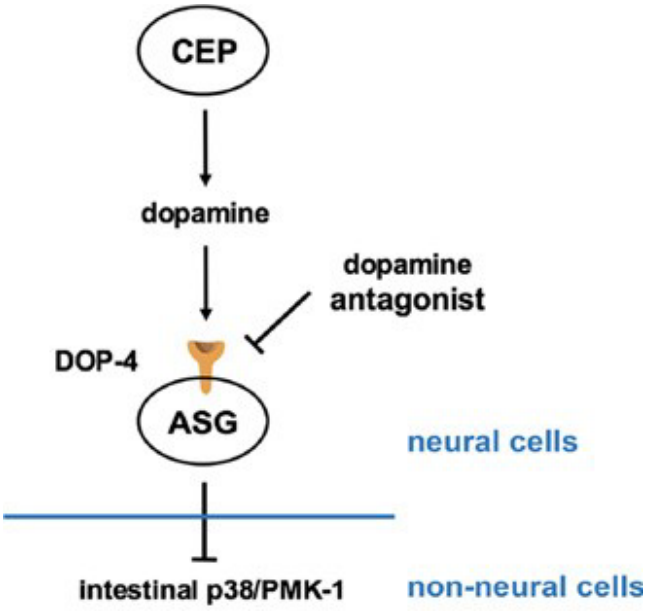

Figure 2. Dopamine release modulates innate response by p38/PMK-1. Dopamine is released by the CEP neuron and binds to DOP-4 receptors on the ASG neuron regulating the intestinal p38/PMK-1 pathway. The pathway can be suppressed by adding dopamine antagonists, which bind to the DOP-4 receptors and prevent the neural response. DOP-4, D1-like dopamine receptor; PMK-1, P38 Map Kinase family member 1. Adapted from Cao & Aballay, 2016.

Besides the dopaminergic system modulating innate immunity, the innate response can be regulated by the adrenergic/octopaminergic/tyraminergic system. Octopamine (OA) and Tyramine (TA) act independently through GPCRs to modulate multiple physiological and behavioral processes in response to external stimuli. The enhanced survival against *P. aeruginosa* challenge in the mutants to *octr-1* (*C. elegans* lacking OCTR-1, a OA receptor involved in negative regulation of innate immune response), *tbh-1* (that promotes decreased OA levels and increased resistance against *P. aeruginosa* infection), *tdc-1* (deletion in L-aromatic amino acid decarboxylase with homology to histidine decarboxylase, resulted in increased tyrosine levels and, improved the resistance against *P. aeruginosa* infection), or both OCTR-1 and TBH [*octr-1*(ok371); *tbh-1*(n3247)] could be due to increased pathogen avoidance because avoidance behavior is part of the *C. elegans* defense response (Sellegounder et al., 2018).

When measuring the expression levels of the immune genes in *P. aeruginosa*-infected *tbh-1*(n3247) animals and comparing the levels to those in infected wild-type or *octr-1*(ok371) animals, all seven *abu* genes tested (*abu-1*, *abu-6, abu-7, abu-8, abu-12, abu-13,* and *abu-15*) and five of the seven PMK-1-dependent genes (C09H5.2, C29F3.7, F08G5.6, F35E12.5, and W03G1.7) were significantly upregulated in *tbh-1*(n3247) animals. These results indicate that an additional pathway(s) that circumvents the necessity of TBH (tyramine-β-hydroxylase enzyme) for OA synthesis might occur, therefore producing small amounts of OA that suppress the gene expression (Sellegounder et al., 2018). Hence, the authors suggested a model in which the octopaminergic immunoinhibitory pathway is tonically active in wild-type animals under normal conditions to maintain immunological homeostasis, probably suppressing unwanted innate immune responses (Figure 3).

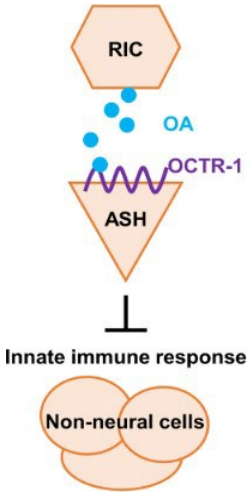

Figure 3. Octopamine is an endogenous antagonist in the innate response in *C. elegans* by signaling in ASH. RIC neurons release OA to OCTR-1 receptors located on ASH neurons, suppressing the innate immune response in non-neural tissues. OA, octopamine; OCTR-1, octopamine receptor 1. Adapted from Sellegounder et al., 2018.

In the octopaminergic immunoinhibitory pathway in *C. elegans*, OA is released from RIC neurons and acts as a ligand of OCTR-1, which functions in the sensory ASH neurons to suppress innate immune responses in non-neural tissues (Sellegounder et al., 2018). In mammals, several neurotransmitters act on the immune system to modify its function (Levite, 2012). In *C. elegans,* serotonin signaling allows animals to respond to changes in their environment by modulating locomotion (Sawin et al., 2000), feeding (Avery & Horvitz, 1990), defecation (Ségalat et al., 1995), and egg-laying (Serafeim & Gordon, 2001). Interestingly, serotonin signaling is also required for *C. elegans* to respond to infection by *P. aeruginosa* PA14 [3,5,6]. Animals that lack *tph-1* are more susceptible to PA14 than wild-type animals. However, in this context, serotonin signaling is not required for the *C. elegans* immune response, and changes in susceptibility of *tph-1* mutant animals are due exclusively to behavioral pathogen avoidance.

4. Pathogen resistance and the p38 MAPK pathway

When the p38 MAPK gene (*pmk-1*) in *C. elegans* is silenced by mutation or RNA interference, the susceptibility to infection by pathogens is increased, which proves the importance of the signaling pathway in the immune response (Bolz et al., 2010). The p38 MAPK signaling pathway constitutes another form of defense presented by *C.*

elegans to protect itself from attacks by pathogens. According to studies the genes involved in the infection caused by pathogens are homologous and will be expressed, with the p38 MAPK pathway and the apoptotic pathway being both the most relevant vias in humans (Morales et al., 2021). The reduction of PMK-1 in mutated *C. elegans* increases the susceptibility to infections by pathogens, suggesting that these genes play an important role in defense of such nematodes (Cohen & Troemel, 2015). *C. elegans* mutants for *esp-2/seq-1* and *esp-8/nsy-1*, related to the p38 MAPK signaling pathway, are more susceptible to death at all stages of development when compared with wild-type, demonstrating its relationship with the conservation of the referred signaling pathway.

Numerous microbial pathogens have been shown to affect and induce a defense response in *C. elegans* epithelial cells. For example, we can highlight *P. aeruginosa*, one of the pathogens extensively studied in *C. elegans* and responsible for causing infection in the worm's intestinal epithelial cells. It is known that *C. elegans* lacks professional macrophages and does not appear to have canonical cytokine and chemokine signaling pathways that recruit phagocytic cells (Cohen & Troemel, 2015). However, the nematode has neighboring cells capable of performing phagocytosis, such as gonad, intestinal, muscular, and hypodermic cells (Wan et al.,2021). Despite this, the worm uses a system-wide signal in response to the infection. When affected by intestinal infection, epithelial cells upregulate antimicrobial peptides releasing detoxifying enzymes and efflux pumps responding to different pathogens (Cohen & Troemel, 2015).

Among the signaling pathways that control the transcriptional response that induces infections in *C. elegans* is the p38 MAPK pathway. In parallel to this signaling pathway, others, such as the pathway regulated by the protection factor bZIP and the transcription factor ZIP-2. According to studies, the pathogenicity of *P. aeruginosa* induces protective transcriptional responses, which are mediated by the p38 pathway and the transcription factor ZIP-2. Inhibition of translation by exotoxin A, secreted by the *P. aeruginosa* bacterium, stimulates the activity of these pathways.

pmk-1 mutants are more susceptible to infections by Gram-negative pathogens

such as *P. aeruginosa, Salmonella enterica,* and Gram-positive pathogens such as *Enterococcus faecalis, Staphylococcus aureus*, and fungal infections caused by *Candida albicans* (Pukkila-Worley & Ausubel, 2012). One of the manners bacteria use to attack *C. elegans* is the deactivation of the translation of its mRNA, which prevents the production of antimicrobial molecules, improving the survival of the bacteria. It is worth mentioning that p38 MAP kinase PMK-1 activity is reduced over time, making the worm more susceptible to death from bacterial infection at an older age.

5. p38 MAPK pathway and nutrition

The C. *elegans* has a nutritional source of non-pathogenic microbes, and their feeding behavior influences its innate immune system (Shivers et al., 2009). Therefore, worms can produce beneficial responses against pathogenic bacteria, enabling studies regarding the inflammatory origins of some pathologies that affect humans (Wu et al., 2019). Both nutrient overload and shortage exert influence on *C. elegans*. The nematode's response to nutrient overload is similar to the response activated by stress in endocrine cells during metabolic diseases in humans, where activation of the MAPK pathway occurs. At the same time, the dietary restriction may extend lifespan by modulating p38 signaling in innate immunity. The lifespan under dietary restriction depends on negative regulation at the basal level of p38-ATF-7 immune response. Its negative regulation is related to reduced insulin signaling via IGF-1, which signalizes reducing food intake and exerting appetite control. Reduced insulin/IGF-1 signaling has been associated with increased longevity in *C. elegans*. Hyperactivation of this pathway is related to an acceleration of aging, activated by nutrients independent of mTORC1 (Wu et al., 2019). A study using sesamin as a nutritional source for *C. elegans* showed an increase in the lifespan mean, via the p38 MAPK pathway (Yaguchi et al., 2014). Nutrition in *C. elegans* is age-dependent in young nematodes as the supplementation with probiotic bacteria and antioxidant agents extend the mean lifespan in addition to improving immunity, while this has not been observed in senescent nematodes (Yaguchi et al., 2014)

6. p38 MAPK pathway and oxidative stress

In addition to its other applications, *C. elegans* has been used as an experimental model to investigate the effect of oxidative stress on innate immune pathways since it is known that stress is highly associated with the activation of the immune pathways in the nematode (Rodriguez et al., 2013). By improving methodologies, studies on the response to oxidative stress through the p38 MAPK signaling pathway in *C. elegans* allow us to precisely examine some of the components involved.

As previously described, PMK-1 is activated in the worm under stress conditions by SEK-1 (MAPKK), an essential upstream activator for PMK-1. The upstream components of SEK-1 differ from components that mediate asymmetric neuronal development and innate immunity, indicating different sensors involved in initiating the immune response and oxidative stress (Inoue et al., 2005). Activation of the p38 MAPK signaling cascade by oxidative stress induces the transcription of genes in the intestine through the PMK-1 pathway, which phosphorylates the transcription factor SKN-1 at its two potential sites represented by Ser residues (Ser-74 and Ser-340) and leads to the translocation of SKN-1 to the gut cell nucleus, promoting the transcription of *gcs-1*, which encodes the phase II detoxification enzyme γ-glutamine cysteine synthase (H. Li et al., 2017). In mammals, *skn-1* is orthologous to Nrf2, a transcription factor that activates the stress response during infections by pathogens (van der Hoeven et al., 2011).

In *C. elegans,* three isoforms of SKN-1/Nrf, SKN-1A, SKN-1B, and SKN-1C were described, which share the same C-terminal region, being different in the N-terminals, in the splicing sites, in their expression and protein sequence editing patterns. SKN- 1A is expressed in all tissues. Eventually, it may produce a processed fragment of SKN- 1 that specifically regulates proteasomal subunit transcription providing an effective method to increase proteasomal activity. SKN-1B has been described as being produced in a single pair of sensory neurons and is involved in food detection. SKN- 1C is produced only in the intestine and is linked to transcriptional activity induced under oxidative stress conditions (Hummell et al., 2021).

The PMK-1 pathway, as seen in the topics above, is regulated by a

phosphorylation cascade, where the TIR-1 leads to the activation of NSY-1 (MAPKKK), which activates SEK-1 (MAPKK) and therefore phosphorylates PMK-1. However, the role of TIR-1 and NSY-1 for SKN-1 activation under stress conditions is still uncertain (Liberati et al., 2004). Some studies suggest that NSY-1 is critical for stress resistance, while another claims that NSY-1 and TIR-1 are expendable (X. Li et al., 2011) (Figure 4).

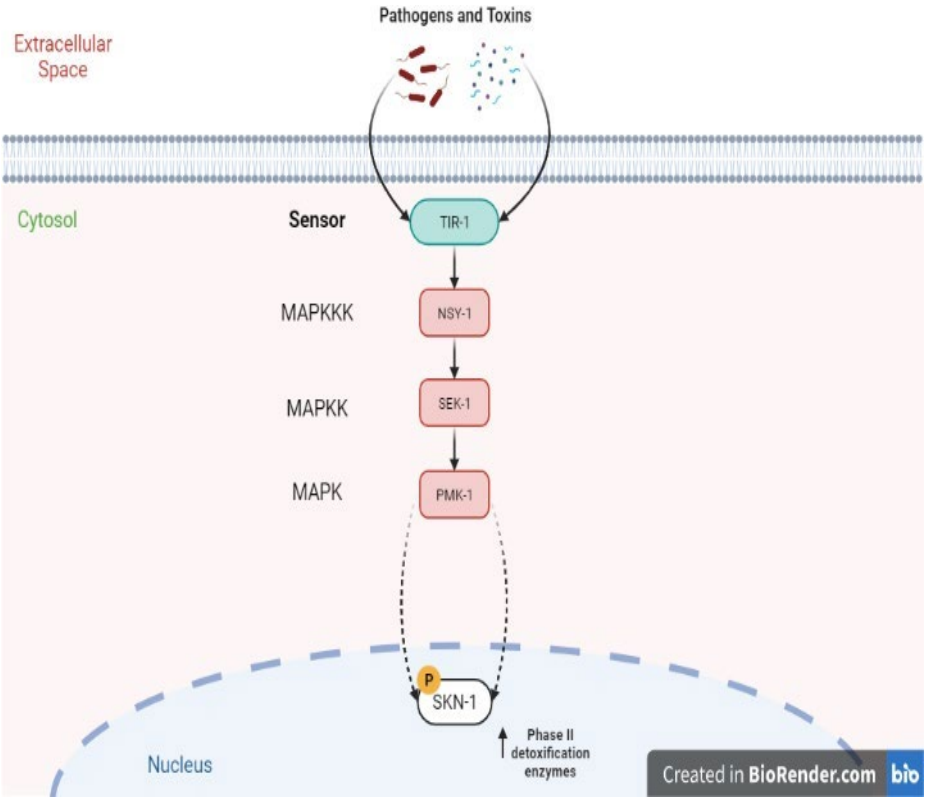

Figure 4. Response of the p38 MAPK signaling pathway in *C. elegans* to oxidative stress. The phosphorylation cascade stimulated by the presence of pathogens and toxins starts from the Toll/IL-1 receptor domain protein (TIR-1), leading to activation of NSY-1 (MAPKKK), which activates SEK-1 (MAPKK) and then PMK-1 (MAPK). The transcription factor SKN-1 is phosphorylated and translocated into the nucleus of intestinal cells enabling the encoding of the phase II detoxification enzyme. MAPK, Mitogen-activated protein kinase; MAPKK, MAPK Kinase; MAPKKK, MAPK Kinase Kinase; NSY-1, Neuronal Symmetry family member; PMK-1, P38 Map Kinase family member 1; SEK-1, SAPK/ERK kinase; SKN-1, transcription factor skinhead-1; TIR-1, Toll/interleukin-1 resistance protein. Source: Authors.

Factors such as infection by pathogens and toxins can produce reactive oxygen species and activate the nuclear translocation of SKN-1 through PMK-1. The context of oxidative stress in *C. elegans* can also be induced by sodium arsenite, paraquat, and t-butyl peroxide, as well as exposure to *P. aeruginosa* and *E. faecalis* (van der Hoeven et al., 2011). It was also reported that worms fed with a specific strain of *Lactobacillus gasseri*

activated SKN-1, which regulated the transcriptional activity of several antioxidant genes compared to worms fed *E. coli* (Mahesh et al., 2021).

7. Conclusion

C. elegans is an animal with innate immunity in the form of molecules and is therefore considered a prime model for studies of molecular immune pathways. Conserved pathways such as insulin/insulin-like growth factor (IIS) and mitogen-activated protein kinase (MAPK) signaling regulate defense against environmental stress and pathogens using p38 MAPK signaling pathways. Thus, the worm has become valuable for studies of immune response pathways, in both up and downregulation consequences. Indeed, they can be evaluated under different types of exposures such as infection, oxidative stress, and nutrition. It is also possible to analyze the connection of pathways and neurons with non-neuronal cells such as intestinal cells, allowing the study of the brain-intestine axis.

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Abbreviations

AGE-1, ageing alteration;
ATF-7, Activating transcription factor 7;
ATF2, Activating transcription factor 2;
bZIP, basic region leucine zipper;
DAF-16, abnormal dauer formation member 16;
DAF-2, abnormal dauer formation member 2;
DOP-4, D1-like dopamine receptor;
ERK, mitogen-activated protein kinase 1;
FOS-1, B-Zip transcription factor homolog 1;
GCS-1, gamma GlutamylCysteine Synthetase;
GCS-1, gamma GlutamylCysteine Synthetase;
GPCRs, G protein-coupled receptors;
HSF1, heat-shock factor 1;
HSPs, heat-shock proteins;
IGF-1, Insulin/insulin-like growth factor;
IIS, Insulin/insulin-like growth factor signaling;
JKK-1, JNK Kinase;
JNK-1, c-Jun N-terminal kinase 1;
KGB-1, Kinase GLH-Binding 1;
KGB-2, Kinase GLH-Binding 2;
KREG-1, KGB-1 regulated gene 1;
LYS-7, Lysozyme 7;
MAPK, Mitogen-activated protein kinase;
MAPKK, MAPK Kinase;
MAPKKK, MAPK Kinase Kinase;
MEK-1, Erk Kinase;
MKP, MAPK phosphatase;
MLK-1, Mixed lineage kinases member 1;
mTORC1, mTOR Complex 1;
NPR-1, NeuroPeptide Receptor family;
NRF2, Transcription factor NF-E2-related factor 2;
NSY-1, Neuronal Symmetry family member;
OA, Octopamine;
OCTR-1, Octopamine Receptor 1;
PMK-1, P38 Map Kinase family member 1;
PRRs, pattern recognition receptors;
SEK-1, SAPK/ERK kinase;
SKN-1, transcription factor skinhead-1;
TA, Tyramine;

TBH Tyramine beta hydroxylase;
TDC, tyrosine decarboxylase 1;
TIR-1, Toll/interleukin-1 resistance protein;
VHP, VH1 dual-specificity phosphatase family;
ZIP-2, bZIP transcription factor family 2.

Chapter 4

Pathogenic microorganisms when applied to the animal model *Caenorhabditis elegans* in a One Health perspective

Douglas Correia de Souza
Francine Côa
Fernanda Karolinne Quaresma Nunes
Ítalo Ian Monteiro da Silva
José Antonio dos Santos Júnior
Laura Portela Siqueira da Costa
Iverson Conrado Bezerra
Priscila Gubert

Abstract

Caenorhabditis elegans is an invertebrate organism that has become an important research platform, emphasizing studies that evaluate pathogens and host interactions. With practical and ethical advantages as a model system, this free-living animal has been establishing itself as a powerful infection system with microorganisms that cause health problems for humans and other animals. In this work, we discussed in a One Health's perspective how a surprisingly diverse variety of pathogens can infect the nematode and how it may constitute a valuable model for studying the pathogenesis of these microorganisms and their products. In addition, research with *C. elegans* and these interactions makes it possible to develop new methods in the search for new antimicrobial agents, diagnostics and develop new therapeutic strategies to promote general health.

1 Introduction

Animal and plant are constantly in contact with microorganisms and other organisms in a complex network of ecological interactions. These interactions affect diverse biological functions and ultimately shape the evolutionary trajectories of these organisms. A good understanding of how microorganisms interact with their hosts directly influences our ability to develop solutions for health problems related to pathogens that harm humans, animals, and plants (ZHANG; HOU, 2013).

Among model organisms used to study these interactions between microbes and

hosts, *Caenorhabditis elegans* stands out due to several unique attributes. It is characterized as a multicellular invertebrate organism, with a completely sequenced genome, non-hazardous, non-infectious, non-pathogenic, and non-parasitic, with a short life cycle and easy maintenance in the laboratory (KIM; FLAVELL, 2020). Furthermore, a large amount of data shows that several developmental, neurological, cell biology, and biochemical processes between the nematode and mammals are highly conserved (HA et al., 2022). *C. elegans* is a small free-living nematode found in temperate regions. It naturally feeds on microorganisms, including bacteria and fungi, and is also a natural host for some pathogenic microorganisms: the bacterium *Microbacterium nematophilum*, the fungus *Drechmeria coniospora*, the microsporidian parasite *Nematocida parisii*, and the Orsay vírus (FÉLIX et al., 2011).

As microorganisms are nematodes' main source of nutrients, the capacity to generate different responses to pathogens and non-pathogens is essential for its survival since certain microorganisms can cause infection and death of the nematode (SAMUEL et al., 2016). The innate recognition of pathogens by *C. elegans* is evident from observations of the animal's phenotypic behavior in the presence of bacteria and their metabolites. Behaviors such as feeding, defecation, egg-laying, and locomotion are affected. Through its sensory neurons, the animal can identify volatile organic molecules produced by pathogens that the nematode has previously contacted, generating the ability to evade these pathogens (ZHANG; LU; BARGMANN, 2005). This biological process was evidenced using the pathogenic strain of *Pseudomonas aeruginosa*, where RNAi pathways proved necessary to transport RNA fragments produced in the pathogen's biofilm to the germ line and later to specific neurons, generating a transgenerational epigenetic response observable in 4 generations (KALETSKY et al., 2020).

Among all the possible uses for *C. elegans*, its applicability in toxicology studies is especially noted, given the fact that it is sensitive to several substances, including heavy metals, organophosphates (compounds of organic phosphates), and pesticides (LEUNG et al., 2008), being widely used for studies involving environmental (YU et al., 2020). In addition, several studies have also demonstrated *C. elegans* as a model for rapid testing of the toxicity of soil and water samples, also as pharmaceutical and

phytochemical compounds (SCHOUEST et al., 2009). The most relevant parameters for toxicological screening are mortality (DENGG; VAN MEEL, 2004), longevity, behavior/movement, feeding, growth, and reproduction (ANDERSON; BOYD; WILLIAMS, 2001)

Despite all the applicability in biotechnological research involving human, animal, and environmental health, its correlation with One Health's concept has not yet been properly explored. Thus, considering the great importance and diverse applications of the nematode for science, the main objective is to review the use of *C. elegans* in studies as a host of several pathogens within One Health's perspective, focusing on the importance of visualizing the concept of health involving humans, animals, and plants in an ecosystem (CENTERS FOR DISEASE CONTROL AND PREVENTION; NATIONAL CENTER FOR EMERGING AND ZOONOTIC INFECTIOUS DISEASES (NCEZID), 2022). The uninterrupted ascension in the development of numerous biotechnologies takes *C. elegans* to an important level for the diffusion and evolution of scientific/social processes and their demands. It is essential to understand the pathogenesis of organisms - which, in turn, requires a suitable host to study the infection process - and the worm almost always meets the most diverse requirements. In this perspective, we highlight the reasons that make *C. elegans* a model with great potential within One Health's concept.

2. *Caenorhabditis elegans* as a bacteria host

The nematode *C. elegans* is recently being developed as a model system to study host-pathogen interactions. *C. elegans* provides a facile and rapid system for studying this interaction as it naturally feeds on bacteria. The pathogenicity of specific strains of bacteria can be directly determined by feeding the nematodes with the bacteria and looking for effects within hours or a few days. The ability to perform forward and reverse genetics in *C. elegans* also lends itself to understanding host pathways that respond to pathogens (for review, see (ABALLAY; AUSUBEL, 2002; ALEGADO et al., 2003; EWBANK; ZUGASTI, 2011) (Figure 1).

Virulence factors produced by bacteria are crucial elements of the pathogenic

attack on the host, overcoming its defenses and establishing the infection. In addition to the ability to harm its host, these factors also involve competition for nutrients and the protection of the pathogen itself. When invading a host, the environment makes the pathogens need quick responses, activating different pathways in response to those activated by the host. The interactions of different bacterial virulence factors with the host *C. elegans* have been investigated. In this article, some of them will be mentioned. It is essential to elucidate these pathogen virulence pathways that generate health problems. It is believed that pathogens have, within their evolutionary courses, gradually added virulence factors in their genetic material according to different antimicrobial and environmental responses, generating the ability to invade and colonize new environments.

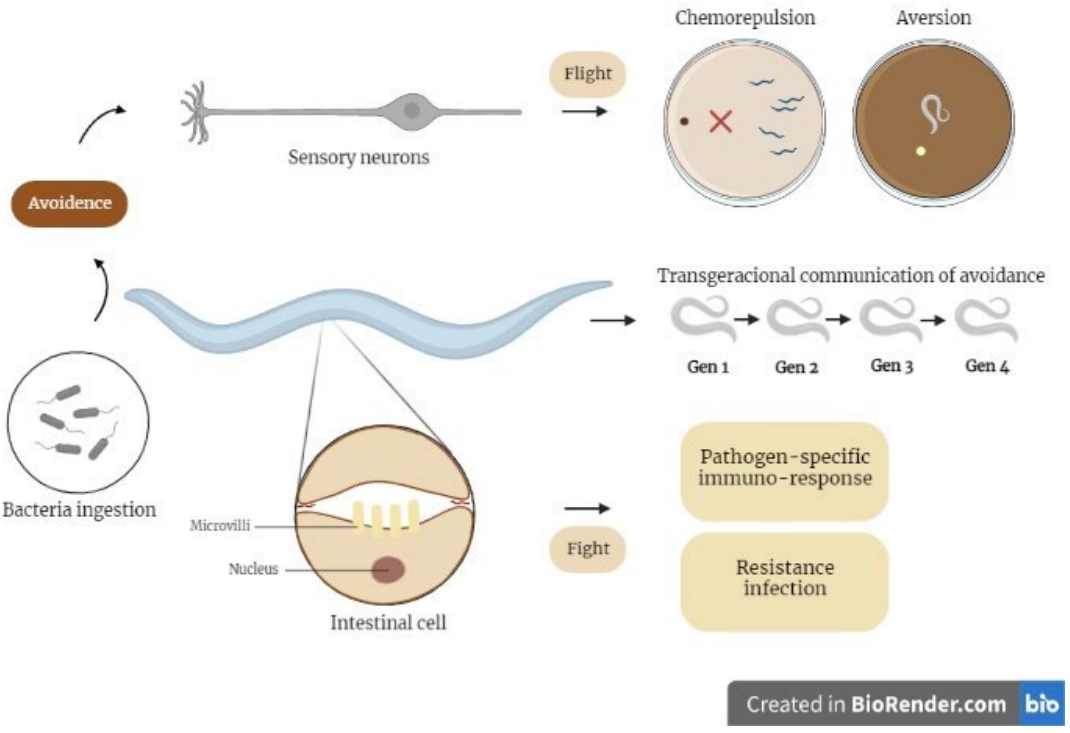

Figure 1: *C. elegans* as bacteria host. After ingestion, *C. elegans* activates pathways for specific pathogenic responses resulting in avoidance stimuli such as chemorepulsion and aversion, fight through immune responses, and stress resistance, in addition to the transgenerational avoidance response.

Thus, the association of *C. elegans* with pathogens is relevant to studying conserved virulence mechanisms used to evade host defenses and attack cells. Studies of *C. elegans* with pathogens may thus elucidate several insights into the evolution of those bacteria and their interaction with cells. Elucidation of the pathways that

participate in host-pathogen interaction provides a unique starting point to identify previously unknown signaling pathways and molecular mechanisms of the host's immune response to bacterial virulence. Understanding how the pathogen interrupts the defense and causes damage and death to the host is fundamental to identifying new therapeutic targets to treat infectious diseases and providing relevant information about its pathogenesis (BEGUN et al., 2005). Here, we compared the human infections by bacteria and the *C. elegans* infection outcomes (Table 1).

Table 1: Human and *Caenorhabditis elegans* infection outcomes in hosting bacteria.

Streptococcus spp.	
Human infection	*S. agalactiae*: newborns and pregnant women infections.
	S. pyogenes are human pathogens that cause invasive and non-invasive diseases such as acute pharyngitis, meningitis, pneumonia, puerperal fever, sepsis, streptococcal toxic shock syndrome, and necrotizing fasciitis.
	S. pneumoniae: colonizer in the upper respiratory tract. Once invasive, it can cause pneumonia, bacteremia, and meningitis.
***C. elegans* infection**	*S. agalactiae*: decreases the average life span and causes body deformations (SHABAYEK; SPELLERBERG, 2018; SILVA, 2020).
	S. pyogenes: causes death within a few hours and is mediated exclusively by hydrogen peroxide, without intestinal colonization (JANSEN et al., 2002).
	S. pneumoniae: kill *C. elegans* by producing hydrogen peroxide (GOOD, 2020; PEREIRA et al., 2022).
Pathogenic *Escherichia coli*	
Human infection	Enteropathogenic *E. coli* (EPEC): fulminant diarrhea, vomiting, dehydration, and fever.
	Enterohemorrhagic *E. coli* (EHEC): diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome.
	Enterotoxigenic *E. coli* (ETEC): diarrhea.
	Enteroaggregative *E. coli* (EAEC): diarrheal conditions. Persistent diarrhea in HIV patients and undernourished children (BOISEN et al., 2020; KAUR; CHAKRABORTI; ASEA, 2010).

	Enteroinvasive *E. coli* (EIEC): cross the intestinal wall through cell destruction and produce severe watery or bloody diarrhea, with mucus and fecal leukocytes, fever, systemic toxicity, cramps, and abdominal pain (PASQUA et al., 2017).
	Uropathogenic *E. coli* (UPEC): causes community-acquired and nosocomial urinary tract infections (UTIs). It requires long-term antibiotic therapy (SCHIFANO et al., 2019).
***C. elegans* infection**	EPEC: paralyzes and worm death dependent on toxins. *hif-1* and *egl-9* are involved in mediating toxin effects. Worm mutants for *sek-1*, *mek-1*, *mev-1*, *pgp-1,3*, and *vhl-1* were more susceptible to EPEC. *daf-2*, *age-1*, and *daf-16* mediated effects of EPEC virulence factors in the worm life span (ANYANFUL et al., 2005).
	EHEC: accumulates in the intestinal lumen, redistribution of specific subcellular actin from the apical site in the basolateral cytoplasm of these cells, followed by damage in the alimentary tract. It impairs the microvillar proteins, known as ACT-5(CHOU et al., 2013; HUANG et al., 2021).
	ETEC: impact worm survival related to MAPK, insulin/insulin-like growth factor pathway in infected animals (TAN et al., 2020; ZHOU et al., 2018).
	EAEC: accumulates in the animal's intestine. Colonization of the distal *C. elegans* intestine.
	EIEC: kill *C. elegans*.
	UPEC: efficiently colonize the intestine and inhibit the host's oxidative response to infection (SCHIFANO et al., 2019).
Shigella flexneri	
Human infection	It promotes invasive infection of the human colon (PIRES et al., 2015).
***C. elegans* infection**	It affects many metabolic pathways involved in generating ATP, causing large accumulation of ROS, damage to cell organelles, leading to autophagy and ultimately cell death (SOMASIRI et al., 2020).

2.1 Caenorhabditis elegans and *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive bacteria part of the natural microbiota of humans and animals. However, when there is some imbalance in the relationship

between the host and the environment with the agent, *S. aureus* proves to be a versatile pathogen capable of causing several diseases such as severe skin infections, soft tissue abscesses, food poisoning, and life-threatening infections such as sepsis, endocarditis, pneumonia and toxic shock syndrome (SIFRI et al., 2003). It has gained significant prominence in studies on the host-pathogen relationship, as treating *S. aureus* infections has become a severe public health problem due to the progressive emergence of generalized antimicrobial resistance. Methicillin-resistant isolates have steadily increased over the past three decades. The pathogen is widespread, whereone-third of the human population carries *S. aureus* in the microflora of the nasopharyngeal epithelium, skin, and intestine. It is one of the leading causes of hospital and community-acquired infections worldwide. It is also the cause of considerable damage to animal welfare and economic paths due to the high incidence and impact of bovine and ovine mastitis in production animals (IRAZOQUI et al., 2010).

Several works on the association of *C. elegans* with different strains of *S. aureus* generated different studies and biotechnological products (BOGAERTS et al., 2010; ESSEBE et al., 2017; SON et al., 2016). When exposed to the pathogen, it is possible to visualize the internal tissue degradation with rapid intestinal colonization, swelling of the anus, and destruction of the intestinal epithelial cells of the worm, leading to death in a few days. The pathogen expresses different and effective virulence factors, which include cytolysins capable of destroying host cells and tissues. Other factors not yet fully elucidated come from thermolabile toxins that cause cell lysis. It has been shown that this process is independent of hemolysin, one of the essential agents produced by the pathogen and inactivated by heat. It has been observed and discussed that host tissue damage is caused by the active pathogen, not by an exacerbated host response (IRAZOQUI et al., 2010; THOMPSON; BROWN, 2021).

S. aureus also mediated wound infection associated with increased *C. elegans* survival. Generally, *S. aureus* decreases *C. elegans'* survival. However, wound mechanisms involved activation of oxidative stress responses, increased calcium signaling, and pathways such as MAPK signaling, oxidative phosphorylation, and phosphatidylinositol signaling. The whole pathogen stress triggered an incitation of

C. elegans survival (KONG et al., 2014; POORANACHITHRA et al., 2021; WANI et al., 2021). *C. elegans* was a crucial tool to reveal the *S. aureus* capture of d-amino acids from hosts to promote in vivo cell-surface remodeling. Such bacterial acting had been only observed under *in vitro* culture conditions (PIDGEON; PIRES, 2017).

2.2 *Caenorhabditis elegans* and *Pseudomonas aureginosa*

Pseudomonas aeruginosa is an aerobic gram-negative, motile oxidase-positive bacteria that oxidizes simple carbohydrates such as glucose (BEHZADI; BARÁTH; GAJDÁCS, 2021). It colonizes soil and aquatic habitats, infecting animals and plants. It is the most frequent cause of infection among non-fermenters. It predominantly affects immunocompromised patients in humans but has also affected immunocompetent patients, often causing chronic panbronchiolitis, cystic fibrosis, and urinary tract infections (WANG et al., 2021).

P. aeruginosa has a classic "secretory" genome, with regulatory genes, such as efflux pumps and other transport proteins, motility, chemotaxis, and genes that regulate metabolic pathways, allowing adaptation in environments with different metabolic states. It has a problematic therapeutic scope due to the significant increase in antimicrobial resistance, which leads to prolonged treatments, sequelae, and increased mortality in affected populations (XIAO et al., 2021; YANG et al., 2021). It has been highlighted in studies of several factors related to virulence and the role of its biofilm in several vertebrate hosts, such as birds and cattle, and in invertebrate models, such as *Drosophila* and *C. elegans* (VASQUEZ-RIFO et al., 2022). It was shown that the culture medium differs in pathogenicity in front of the nematode, once cultivated in a simpler medium, the pathogen caused the worm's death in three days, where the death was related to the accumulation of the bacteria in the intestine of the animal. When grown in more nutrient-rich media, *C. elegans* dies within hours of exposure. These points indicate the plasticity of pathogen gene expression in different environments and how this relationship exists with different eukaryotic cells (TAN; MAHAJAN-MIKLOS; AUSUBEL, 1999).

C. elegans can change several behavioral, stress responses, and innate immune pathways to counterparts in *P. aeruginosa* infection. The volatile molecule 1-undecene

produced by *P. aeruginosa* activates *C. elegans* odor sensory neurons inducing both flight (avoidance behavior) and fight (pathogen-specific immune response) responses in worms. The immune pathways implicated in infection resistance comprise to *pmk-1*/P38 MAP kinase pathway, the *fshr-1*/G protein-coupled receptor (GPCR) pathway, and the *zip-2* pathway. Indeed, it has been suggested that *P. aeruginosa* can induce ribosome degradation and helix 69 (H69) cleavage of host ribosomes, which can impair host translation and block antibacterial responses (VASQUEZ-RIFO; RICCI; AMBROS, 2020).

2.3 *Caenorhabditis elegans* and *Streptococcus spp.*

Streptococcus genus comprises complex species that act as pathogens, are Gram-positive, visualized as spherical or ovoid, and often arranged in chains or pairs. They colonize environments and are found naturally in the skin, mucous membranes, and respiratory tract of humans and animals. Several *Streptococcus spp* are important pathogens, as the most frequently reported are *S. agalactiae, S. pyogenes,* and *S. pneumoniae*. These species have been highlighted especially due to emerging antimicrobial resistance, virulence, and zoonotic potential, with economic losses and social impacts (GARSIN et al., 2001; GOOD, 2020; KAITO et al., 2020). *Streptococcus agalactiae* is a widespread pathogen associated with infections in newborns and pregnant women. Transmissions between humans and herds free of infections have been demonstrated, where the contact spread was via the fecal-oral route between the environment, humans, and animals. This demonstrates how neglected reservoirs contribute to different routes of transmission and/or contamination of this pathogen. In studies with *C. elegans* the pathogen decreases the average life expectancy and causes body deformations (SHABAYEK; SPELLERBERG, 2018; SILVA, 2020).

Streptococcus pyogenes are human pathogens that cause invasive and non-invasive diseases such as acute pharyngitis, meningitis, pneumonia, puerperal fever, sepsis, streptococcal toxic shock syndrome, and necrotizing fasciitis. However, it is rarely isolated in animals. Once *S. pyogenes* infect *C. elegans*, the nematode dies within a few hours, demonstrated in both solid and liquid media. Death by *S. pyogenes* is

mediated exclusively by hydrogen peroxide and can be inhibited by catalase. The main exotoxins of this pathogen are not involved in the process of worm death, as confirmed by the use of toxin inhibitors and mutant strains in the membrane-perforating enzymes (SLO and SLS) and proteinases (SPE-B). Furthermore, no accumulation of *S. pyogenes* was observed in *C. elegans*, which excludes the involvement of infectious processes with intestinal colonization (JANSEN et al., 2002).

Streptococcus pneumoniae act as a colonizer in the upper respiratory tract, where colonized individuals serve as a reservoir for the pathogen, facilitating its transmission in the community. Once invasive, it spreads to the lungs, causing pneumonia. In other organs, *S. pneumoniae* can cause serious diseases such as bacteremia and meningitis with significant morbidity and mortality. Several strains were studied with the nematode, whose the most virulent induced rapid death of the animal in a few hours. It has been demonstrated that *S. pneumoniae* can also kill *C. elegans* by producing hydrogen peroxide. Hydrogen peroxide-mediated killing may represent a common virulence mechanism in Gram-positives (GOOD, 2020; PEREIRA et al., 2022).

2.4 *Caenorhabditis elegans* and pathogenic *Escherichia coli*

E. coli are versatile non-spore forming facultative anaerobes rod-shaped Gram-negative bacteria that are generally motile by peritrichous flagella. *E. coli* is revealed as a harmless commensal component of the gut microbiota in mammals which plays a physiological role in the organism's functioning. It may also be a pathogen that expresses several groups of virulence factors. It is a bacterium with a high level of genetic alterations that occur naturally and randomly in the environments it inhabits. These changes make *E. coli* such microorganisms with highly adaptive characteristics in which specific virulence attributes are dynamically expressed and added to their genome. The consequence is the extraordinary capacity to infect new niches and allows them to cause a wide spectrum of diseases. In addition, it is essential to give attention to the dissemination of genes between strains of *E. coli* that make these bacteria resistant to antibiotics, as this phenomenon has increased exponentially since the early 2000s. In addition, as a bacterium propagated by water, it makes a valuable marker for fecal pollution and food and water safety (ITHETE et al., 2013; KAPER; NATARO;

MOBLEY, 2004).

Certain groups of virulence factors are known as pathotypes. *E. coli* presents several groupings based on the genetic or phenotypic characteristics of the infection caused by the pathogen. Pathogens can be grouped by shared antigens, such as those that express the O antigen (lipopolysaccharide, LPS) and the H antigen (flagellar) that define Serogroups (O antigen only) or Serotypes (O and H antigens). Six pathotypes are described within intestinal origin bacteria: enteropathogenic *E. coli* (EPEC), enterohemorrhagic, *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC). Human pathotypes are classically differentiated into nonpathogenic *E. coli* and extraintestinal pathogenic *E. coli* (ExPEC). ExPEC is classified as uropathogenic *E. coli* (UPEC), sepsis-causing *E. coli* (SEPEC), and neonatal meningitis-associated *E. coli* (NMEC). Avian pathogenic *E. coli* (APEC) is a critical pathotype that causes infections and has its biological reservoirs in birds and is associated with respiratory infections and avian septicemia (GLADSTONE et al., 2021; PERCIVAL; WILLIAMS, 2014).Like other mucosal pathogens, pathogenic *E. coli* evade host defenses by colonizing and multiplying, thus causing damage through multi-step pathogenesispatterns. They can also act as opportunistic pathogens when they eventually reachoutside their natural habitat causing diverse extraintestinal infections. Despite being a microorganism widely studied in its genetic and physiological aspects, the mechanisms by which *E. coli* maintains a well-structured symbiosis in different niches are still not well characterized. Unlike most pathogenic *E. coli*, which are extracellular, EIEC can enter and replicate within different cells, such as macrophages and epithelia. Several diseases in animals caused by different pathotypes such as EPEC, EHEC, and ETEC are reported, where the same virulence factors of the strains present in humans are often expressed (PALANIAPPAN et al., 2006; ROBINS-BROWNE et al., 2016).

2.4.1 Enteropathogenic *Escherichia coli* (EPEC)

Enteropathogenic *E. coli* are non-enterotoxin-producing pathogens that can adhere to intestinal epithelial cells and demonstrate Shigella-like invasiveness. It causes conditions that range from subclinical infection to fulminant diarrhea,

vomiting, and fever. It is a major contributor to mortality and morbidity in developing countries. It affects dangerously and with high mortality rates in children up to 2 years of age and is associated with dehydration. In recent years, due to several new molecular and cellular biotechnological processes, the pathogenesis mechanisms of the virulence factors of this pathotype have been better understood. The dependence on the structural injury to induce EPEC virulence is where the bacterium makes contact with the apical plasma membrane, developing localized damage. From this connate, there is the formation of actin-rich structures and cytoskeletal rearrangements (CHEN; FRANKEL, 2005; CLARKE et al., 2003).

C. elegans is already widely used as a platform to evaluate the pathogenesis of these pathogens, where EPEC paralyzes and kills the worm. In this way, it can be valuable for elucidating new virulence factors that may arise and how important genes in mammals regulate and mediate these pathways. Both paralysis and death of the animal do not require direct contact, demonstrating the need for the toxin secreted in the medium for its effectiveness. The requirement for the tryptophanase enzyme, which catalyzes the production of indole and other molecules from tryptophan, was evidenced. In *C. elegans*, several genes, when mutated, alter the pathogenesis of EPEC strains in the animal, such as *hif-1* and *egl-9*, which are involved in mediating toxin effects. On the other hand, worm mutants for *sek-1, mek-1, mev-1, pgp-1,3,* and *vhl-1* were more susceptible. That suggests a crucial role in protecting the worms against EPEC infection. In addition, genes linked to animal life expectancy, including *daf-2, age-1*, and *daf-16* mediated the effects of EPEC virulence factors in the process (ANYANFUL et al., 2005).

2.4.2 Enterohemorrhagic *Escherichia coli* (EHEC)

Enterohemorrhagic *E. coli* are pathogens colonizing the gastrointestinal tract, causing diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. EHEC are classified into three groups according to determining virulence factors, such as plasmid pO157 and locus of enterocyte effacement (LEE) products. Another group, the Shiga-type toxin-producing EHEC (Stx) is composed of subunits A, which inhibits protein synthesis and causes apoptosis, and subunit B, which mediates, together with

globotriaosylceramide-3(Gb3), the pathogenesis of these virulence factors and pathways involved. Gb3 expression varies across tissues and cells, varying at different levels. Renal glomerular endothelium is a tissue that expresses high levels of Gb3. Thus, Stx-producing EHEC infections result in renal failure, Thrombocytopenia, and Microangiopathic hemolytic anemia (NGUYEN; SPERANDIO, 2012; WELINDER-OLSSON; KAIJSER, 2005).

In *C. elegans*, EHEC accumulates in the intestinal lumen, attaching to and mediating pathways with LEE. Besides, EHEC causes in the intestinal cells of the nematode the redistribution of specific subcellular actin from the apical site in the basolateral cytoplasm of these cells, followed by damage in the alimentary tract of the worm. In addition to these microvillar proteins, known as ACT-5, structuring the central bundle that supports the physiological and structural morphology of microvilli, they also play a role in the rotation of these structures, making them highly dynamic. This distortion caused by EHEC and its association with ACT-5 can be used as a biomarker for the study of microvillar deletion using *C. elegans*. There is currently no vaccine or targeted therapy, and antibiotics use is controversial because the mechanisms involved are unclear. In fact, some drugs can negatively impact the prognosis of the infected person by altering the pathogen's expression of other factors (CHOU et al., 2013; HUANG et al., 2021).

2.4.4 Enterotoxigenic *Escherichia coli* (ETEC)

Enterotoxigenic *E. coli* are pathotypes that produce heat-stable toxins (STp and STh) and heat-labile toxins (LT), in addition to the expression of colonization factors (CF). CF's participate in the adhesion cascade in the intestinal mucosa. Thus, ETEC is an important cause of diarrhea in several countries. Different virulence factors, such as enterotoxins, colonization pathways, and O antigens, characterize this group of lactose fermenters differently from other diarrheal pathotypes. Rational treatment with antimicrobials, together with intense rehydration, are part of the excellent clinical conduct in diarrheal cases, so attention should be paid to the growing increase in antibiotic resistance in infections caused by ETEC, mainly due to incorrect antimicrobial use, since in healthy patients it may not be necessary to use any agent (DUAN et al., 2019; RODAS et al., 2011).

ETEC causes *C. elegans* death. It has been known that pathways such as MAPK, insulin/insulin-like growth factor pathway, and others affect the average life expectancy of the infected animal. The association of *C. elegans*/ETEC and probiotic agents allowed research and the creation of new therapeutic agents for diarrhea in humans and animals. *C. elegans* was used to prove that those probiotic agents regulated the expression of ETEC enterotoxins (TAN et al., 2020; ZHOU et al., 2018).

2.4.5 Enteroaggregative *Escherichia coli* (EAEC)

Enteroaggregative *E. coli* is a heterogeneous category of emerging enteric pathogens associated with diarrheal conditions. It is characterized by the ability to produce virulence factors that generate aggregative adherence (AA), form biofilms, and do not express thermostable and thermolabile toxins. Excellent colonizers with the AA's can adhere to cells, other bacteria, and materials such as glass. This genetic heterogeneity makes the robust elucidation of these pathogenic processes difficult; another point is that EAECs are usually isolated from patients with characteristic clinical pictures, but it is also isolated from asymptomatic individuals. The incidence of persistent diarrhea has been highlighted in people affected by HIV and children in a state of malnutrition in developed and developing regions (BOISEN et al., 2020; KAUR; CHAKRABORTI; ASEA, 2010).

The association of EAEC with *C. elegans* is being used as a platform for elucidation and studies of aspects of the pathogenesis of these bacteria. Several pathways that affect mammalian cells also generate damage in nematode cells. When infected, it is possible to visualize the colonization of the distal *C. elegans* intestine and the accumulation of the pathogen in the animal's intestine. The persistence of more pathogenic strains in the intestine was also observed, causing nematode death within a few days after the onset of infection (BLANTON et al., 2018; HWANG et al., 2010).

2.4.6 Enteroinvasive *Escherichia coli* (EIEC)

Enteroinvasive *E. coli* are pathotypes that cause health problems in humans and animals. They cause watery or bloody diarrhea, with mucus and fecal leukocytes, in addition to fever, systemic toxicity, cramps, and general abdominal pain. EIEC shares

virulence genes with *Shigella* spp, which makes their pathogenic aspects similar. EIEC is responsible for about 20% of cases of bloody diarrhea outbreaks in developing countries. This pathotype, like *Shigella*, can invade the intestinal epithelium, where it produces dysentery-like conditions. These proteins that mediate cell invasion form the needle-like injection apparatus, a system bacteria use to transport proteins to the plasma membrane and inject toxins into the host cell. After the invasive process, there is cell multiplication, intracellular and intercellular dissemination, leading to the death of the host cell (PASQUA et al., 2017).

Due to the phenotypic and genetic similarity of EIEC to *Shigella*, studies comparing the pathogenicity of these pathogens in *C. elegans* can create ways to understand better the processes involved in these pathways. EIEC strains kill *C. elegans* statistically more than the Shigella strains tested, where both colonized the animal's intestine (FUNG et al., 2015).

2.4.7 Uropathogenic *Escherichia coli* (UPEC)

Uropathogenic *E. coli* is the leading cause of community-acquired and nosocomial urinary tract infections (UTIs), representing substantial medical costs and morbidity worldwide. UPEC has been associated with acute, chronic, and recurrent infections that require long-term antibiotic therapy (SCHIFANO et al., 2019). UPEC produces numerous virulence factors, including various adhesins, iron chelators, capsule-forming polysaccharides, and toxins (e.g., hemolysin, necrotizing cytotoxic factor 1), which allows UPEC to colonize and manipulate the innate immune response of the host (JOHNSON; RUSSO, 2002). The ability of UPEC to invade and multiply in host epithelial cells and form biofilms also increases the virulence and persistence of UPEC in the urinary tract (CHEN et al., 2012).

It has been reported that free-living nematodes can serve as carriers or vectors of human enteric pathogens from soil resources, and these nematodes have been shown to be resistant to free chlorine and offer protection to ingested pathogens against chemical sanitizers (CALDWELL et al., 2003). Therefore, *C. elegans* could serve as an *in vivo* model to distinguish different virulence behaviors among uropathogenic *E. coli*. Study results showed that urinary strains can kill *C. elegans*, and one of the

clinical isolates tested was able to efficiently colonize the intestine and inhibit the host's oxidative response to infection (SCHIFANO et al., 2019).

2.4.8 Caenorhabditis elegans and *Shigella flexneri*

Shigellosis (bacillary dysentery) is a highly invasive infection of the human colon that is responsible for approximately 190 million cases and 66,000 deaths each year (PIRES et al., 2015). Researchers have recently begun using *C. elegans* as a potential animal model to study *Shigella* pathogenesis. The worm is an attractive candidate for studying its pathogenesis for several reasons (GEORGE et al., 2014): the intestinal cells of *C. elegans* share morphological similarities with human intestinal cells. Also, the human innate immune system shares many characteristics with the immune system present in *C. elegans*, suggesting that the nematode may use similar response mechanisms to counter a bacterial infection as its human counterpart. Finally, the transparent anatomical structure, availability of self-fertilizing hermaphrodites, fast regeneration time, and the requirement of a range of bacterial virulence factors to induce pathogenesis make *C. elegans* an ideal intestinal model for studying the pathogenesis of *Shigella*.

The presence of *Shigella* in *C. elegans* could potentially lead to severe alterations to many metabolic pathways involved in generating ATP, thus reducing the ability ofthe worms to mount a strong defense. There is also the hypothesis that a considerable accumulation of ROS within the worm cells would cause extensive damage to cell organelles, leading to autophagy and, ultimately, cell death. The overall damage to the cells, most likely the intestinal cells, may cause the eventual death of the worm.

It is not yet clear which specific mechanism(s), if any, are utilized by *Shigella* to bring about such drastic changes within the worm. However, with more studies using *C. elegans* as a model host, it may be possible to investigate and elucidate which mechanisms cause the pathogenesis (SOMASIRI et al., 2020).

2.5 *Caenorhabditis elegans* and other bactérias

Many other Gram-negative and Gram-positive bacteria have been identified as *C. elegans* pathogens, among them (HUFFMAN et al., 2004): *Aeromonas hydrophila*

(COUILLAULT; EWBANK, 2002), *Burkholderia pseudomallei* (GAN et al., 2002; MAHAJAN-MIKLOS et al., 1999; O'QUINN; WIEGAND; JEDDELOH, 2001; TAN; MAHAJAN-MIKLOS; AUSUBEL, 1999), *Salmonella typhimurium* (ABALLAY; YORGEY; AUSUBEL, 2000; LABROUSSE et al., 2000), *Serratia marcescens* (KURZ, 2003; MALLO et al., 2002), *Yersinia pestis* (DARBY et al., 2002), *Yersinia pseudotuberculosis* (DARBY et al., 2002), *Bacillus Thuringiensis* (MARROQUIN et al., 2000), *Enterococcus Faecalis* (Garsin *et al.*, 2001; Sifri *et al.*, 2002), *Microbacterium nematophilum* (HODGKIN; KUWABARA; CORNELIUSSEN, 2000).

2.5.1 *Caenorhabditis elegans* and *Bacillus thuringiensis* (Bt)

Bacillus thuringiensis (Bt) is a pathogenic bacteria that *C. elegans* is likely to encounter naturally in the soil. The pore-forming Crystal (Cry) toxins made by Bt are recognized as the dominant virulence factor in this host-pathogen interaction. Forward genetic screens for *C. elegans* mutants resistant to the Cry toxin, Cry5B, have identified a host carbohydrate structure that promotes pathogenesis.

Cry toxicity is directed against intestinal cells of *C. elegans* and leads to vacuole formation, pitting, and eventual degradation of the intestine following Cry exposure (MARROQUIN et al., 2000). The ability to intoxicate *C. elegans* with Cry toxins allows for the identification of host factors that are required for the intoxication process as well as host factors that defend against the pore-forming toxin. For example, isolation of *C. elegans* mutants (and subsequent cloning of genes) resistant to a Cry toxin reveals host factors used by the toxin for the pathogenic process. Investigations with Cry5B, a host carbohydrate structure, in *C. elegans* show a promising beginning in helping to elucidate host-toxin and host-pathogen interactions (HUFFMAN et al., 2004).

2.5.2 *Caenorhabditis elegans* and Enterobacter cloacae SBP-8

Enterobacter cloacae, an opportunistic nosocomial pathogen, is reported to possess different virulence factors that could potentially influence its pathogenesis. *E. cloacae* SBP-8 progressively colonized the intestine of *C. elegans*. It induced cell death (as assessed through DNA damage), reproductive defect, and reduction of lifespan. Other factors were also observed in infected *C. elegans*: significant anterior pharyngeal

distention, altered egg arrangement with internal egg hatching, reduction of the brood size, mitochondrial damage, oxidative stress, septic shock, and stimulation of immune response. *C. elegans* immunity was probably activated through Tol-1/p38 MAPK pathway. These results demonstrate the pathogenic potential of *E. cloacae* SBP-8 and suggest the suitability of *C. elegans* as a model organism for studying its pathogenesis (KHAN et al., 2020).

2.5.3 *Caenorhabditis elegans* and *Corynebacterium striatum*

Corynebacterium striatum is now included among multidrug-resistant (MDR) pathogens increasingly associated with health-care-associated infections (HAIs) suchas sepsis, endocarditis, meningitis, osteomyelitis, surgical wounds, and urinary and pulmonary infections in immunocompromised and immunocompetent patients, including patients using invasive medical devices (MASUDA et al., 2018). The nematode *C. elegans* has been used as a model host to study the pathogenicmechanisms of several Gram-positive and Gram-negative human pathogens, including the potentially toxigenic corynebacterial species: *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* (ANTUNES et al., 2016).

In studies using *C. elegans* as a host model for infection caused by *Corynebacterium striatum*, the results showed: hemotaxis of nematodes towards *C. striatum*, nematode death (>60%), deformed morphology of the anal region, internal egg hatching and star formation in *C. elegans* nematodes. In view of these results, it was found that *C. striatum* exerts virulence for *C. elegans* (SOUZA et al., 2019).

3. *Caenorhabditis elegans* as a fungus host

Pathogenic fungi are a huge global concern because they are associated with several serious diseases. One billion people are affected by fungal pathogens yearly, and about 500 fungal species are linked with human pathologies (SISCAR-LEWIN; HUBE; BRUNKE, 2022). Therefore, understanding the impacts and behavior of fungus in the organisms is crucial to developing more suitable strategies to control these diseases.

Invertebrate models have been exploited as a strategy to investigate fungal

pathogenesis. Besides the low cost and physiological simplicity, their innate immune mechanisms share evolutionary conservation with humans, which permits obtaining insights about virulence factors, especially in determining virulence genes. *C. elegans* is one of the invertebrate organisms that has become a promising model not only to assess fungal virulence and host defense but also to support the screening and to test the efficacy of novel therapeutic targets (BREGER et al., 2007; MYLONAKIS; ABALLAY, 2005; PUKKILA-WORLEY; AUSUBEL; MYLONAKIS, 2011). During this evaluation, nematodes ingest the fungal pathogens instead of *E. coli* bacteria, and the infection is established in the worm gut, which can suffer distention (BREGER et al., 2007). *C. elegans* has been used to assess infections by the filamentous fungus *Drechmeria coniospora*, which naturally infect nematodes in the environment, but human pathogenic filamentous fungi are also the subject of study. It is worth mentioning that there are some experimental challenges because worms avoid eating conidia, making the infection process difficult. In addition, the protocol for separating the non-ingested conidia from worms is not well established (AHAMEFULE et al., 2021). In Table 2 and the following items, we have summarized the great breakthrough of using the *C. elegans* model for fungal infection and compared it with human fungal effects.

Table 2: Fungal infection impairments in humans and *C. elegans*.

***Candida* spp.**	
Human infection	It causes biofilms formation on medical implants such as catheters and heart valves (KABIR; HUSSAIN, 2009). *C. albicans* can colonize the skin, oral cavities, and vagina or penetrate the blood, causing infections (MAVOR; THEWES; HUBE, 2005).
***C. elegans* infection**	The infection comprises swelling in the vulvar region, deformation in the post anal region and intestinal, and affects fertility (FEISTEL et al., 2019).

***Aspergillus* spp.**	
Human infection	*A. fumigatus*: opportunistic pathogen. Cause mycoses and is resistant to drugs, such as azole, amphotericin B, and echinocandin (ASHU et al., 2018; BEER et al., 2018; JIN, 2012; PRIGITANO et al., 2019).
	A. fumigatus and A. flavus: necrosis and lung tissue inflammation (WILLGER et al., 2009).
***C. elegans* infection**	*A. fumigatus:* 80% of nematodes infected died after three days. It disrupted the epidermis cuticles. Indeed, worm movements were also hampered because of the adhesion of hyphae on cuticles (AHAMEFULE et al., 2020).
***Cryptococcus neoformans* spp.**	
Human infection	Life-threatening meningoencephalitis in immunocompromised patients (BICANIC; HARRISON, 2004).
***C. elegans* infection**	It killed the nematodes. The polysaccharide capsule and genes are associated with signal transduction pathways, such as GPA1, PKA1, PKR1, and RAS1, as well as laccase production (LAC1) and the α-mating-type gene (MYLONAKIS et al., 2002).
***Paracoccidioides* spp.**	
Human infection	It causes granulomatous changes in the nose, sinuses, andskin. Affect the gastrointestinal tract, central nervous system, and skeletal system (BOCCA et al., 2013).
***C. elegans* infection**	*P. brasiliensis* and *P. lutzii* could not be ingested by *C. elegans* due to their large size and irregular shape and, therefore, could not cause infection. However, the mere exposure to the fungi causes an immune response in *C. elegans*: increased *cnc-4*, *nlpl-27*, and *nlp-31* expression (SCORZONI et al., 2018).

3.1 *Caenorhabditis elegans* and *Candida* spp.

Candida, Cryptococcus, and *Aspergillus* are the most common fungus that causes infections in humans. More than 200 ascomycetous yeasts are classified into the genus *Candida*. However, *Candida albicans* is the most isolated species associated with infections in the global healthcare system. *Candida* spp. infections are frequent in hospitals in patients with skin lesions or the biofilms formed on medical implants such as catheters and heart valves (KABIR; HUSSAIN, 2009). Yeast cells can infect organs such as the kidney, heart, spleen, and brain, causing fungemia and life-threatening septicemia.

Among the *Candida* spp, *Candida albicans* is responsible for at least 50% of cases of invasive candidiasis (KULLBERG et al., 2011). *C. albicans* is an opportunist fungus found in the gastrointestinal flora of humans. However, it can colonize the skin, oral cavities, and vagina or penetrate the blood, causing infections (MAVOR; THEWES; HUBE, 2005). *C. parapsilosis*, *C. glabrata*, *C. krusei* and *C. tropicalis* are other opportunistic pathogens. It is known that *C. parapsilosis* can form biofilms on medical implants, being more common in the pediatrics population, while *C. glabrata* is the most common cause of vulvovaginal candidiasis (ABI-SAID et al., 1997). *C. tropicalis* is usually associated with cancer patients; in this case, the mortality rates can be as highas 70% (NUCCI; COLOMBO, 2007).

The scientific community encouraged the adoption of *C. elegans* to discover new bioactive compounds against several types of Candida and to understand its infection pathogenesis. To assess the infection of Candida in *C. elegans*, several markers have been proposed as swelling in the vulvar region, deformation in the post anal region, and intestinal. Candida can also affect *C. elegans* fertility, which is a challenging endpoint to be monitored in mammalian and insect models (FEISTEL et al., 2019). *C. elegans* has also been useful in evaluating the importance of hyphal formation in *C. albicans* pathogenesis, despite there being limitations in this method because the yeast form can kill *C. elegans* (BREGER et al., 2007; PUKKILA-WORLEY; AUSUBEL; MYLONAKIS, 2011).

RIM101 (required for alkaline-induced hyphal growth), *NRG1* (a transcriptional repressor of hyphal genes that act with TUP1 gene), *CAS5* (a transcription factor that regulates cell adhesion, and stress response which acts with Ada2p to promote cell

wall integrity), and *ADA2/CAS3* (transcriptional co-activator involved in cell wall integrity, stress responses, and some metabolic processes), are important genes for both hyphal formation in vivo and the killing of *C. elegans* (PUKKILA-WORLEY et al., 2009). In addition, it was reported that they are also involved in the reproductive capacity of *C. elegans* when infected by *C. albicans* virulence (FEISTEL et al., 2019). In this study, it was demonstrated that infection with deletion mutants of *CAS5*, *RIM101*, and *CEK1* (a kinase required for the yeast-hypha transition) promoted a significantly more off-spring than infection with wild-type, being cas5ΔΔ and cek1ΔΔ the most affected, suggesting that these two mutants are avirulent in *C. elegans*.

3.2 Caenorhabditis elegans and Aspergillus spp.

Aspergillus spp. is a filamentous fungus widely distributed in the environment, especially in the soil, where it is responsible for recycling carbon and nitrogen. However, they are frequently observed in humans and animals, which can cause pathogenicity in immunocompromised organisms or with pre-existing lung pathologies, such as tuberculosis and tumors. In fact, humans are exposed daily to 100 - 1,000 airborne conidia, which can reach and colonize the lung alveoli due to their small size (VAN DE VEERDONK et al., 2017).

Aspergillus spp. can easily adapt to a huge range of environmental conditions and several substrates, in addition to presenting remarkable phenotypic plasticity. Several clinical manifestations in humans are commonly caused by *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus nidulans*, and *Aspergillus terreus*. However, the most severe infection of global incidence is associated with *A. fumigatus*. *A. fumigatus* is an opportunistic pathogen that causes mycoses and shows resistance to classical drugs, such as azole, amphotericin B, and echinocandin (ASHU et al., 2018; BEER et al., 2018; JIN, 2012; PRIGITANO et al., 2019). In addition, *A. fumigatus* and *A. flavus* are known for causing necrosis and inflammation of the lung tissue (WILLGER et al., 2009). In fact, invasive aspergillosis is associated with 90% of mortality cases in transplant patients with hematopoietic stem cells (CARVALHO-DIAS et al., 2008).

C. elegans has been currently applied as a host for understanding the *in vivo*

pathological aspergillosis. Since *A. fumigatus* is one of the leading causes of airborne fungal pathogens, it is the fungi that have been more evaluated in *C. elegans*. Okoli and Bignell (2015) were the first to report the possibility of studying the pathogenicity of *A. fumigatus* in *C. elegans*. They observed that 80% of nematodes infected by *A. fumigatus* died after three days, but the virulence factors involved in this death were not identified. Ahamefule et al (2020) noticed that the conidia growth of *A. fumigatus* led to the death of nematodes, as well as disruption of epidermis cuticles. Indeed, nematode movements were also hampered because of the adhesion of hyphae on nematode cuticles. In addition, they found that using azole drugs can be a strategy to prevent conidial germination in a magnitude higher than amphotericin B. Wei et al (2022) investigated the effects of *A. fumigatus* infection and found that the sat1 gene contributes to the fungi virulence (WEI et al., 2022). Experiments with a mouse model were also performed and confirmed that deleting *sat-1* can be a good option for reducing virulence because they observed a decrease in mortality rate, fungal load, and lesion severity in *sat-1* disrupted strains. The application of fluorescence strains has also been important to elucidate some aspects concerning aspergillosis. Using the Af293-dsRed strain, it was observed that hyphal filamentation could emerge from any part of the nematode body instead of the tail region, as was previously reported (AHAMEFULE et al., 2020; OKOLI; BIGNELL, 2015). Recently, *C. elegans* was crucial to discovering virulence factors of *A. fumigatus*, for example, α-(1,3)-glucan synthase, iron transporter, melanin pigmentation, Zn2Cys6-type transcription factor, and mitochondrial thiamine pyrophosphate transporter, when mutant strains for these components were used (AHAMEFULE et al., 2021).

Therefore, these findings demonstrated that *C. elegans* can be a valuable tool to elucidate the mechanisms involved in fungal infections. Besides, it is very useful for screening and identifying novel drugs and compounds against pathological fungus.

3.3 *Caenorhabditis elegans* and *Cryptococcus* spp.

Cryptococcus is the most prevalent pathogens in the genus belonging to the *C. neoformans* spp. complex (*C. neoformans* and *C. deneoformans*), which is a frequent cause of life-threatening meningoencephalitis in immunocompromised patients (BICANIC;

HARRISON, 2004). The *C. neoformans* spp. complex can be isolated from soil, and bird droppings and infection can occur via inhalation of spores, which may lead to mild symptoms or deadly lung and central nervous system infections. Unlike *C. neoformans* spp. complex infections, infections by *C. gattii* spp. complex (*C. gattii*, *C. bacillisporus*, *C. deuterogattii*, and *C. decagattii*) are rare and can occur in immune-competent individuals. Recent studies showed that *C. elegans* could use some *Cryptococcus* spp., such as *C. laurentii* and *C. kuetzingii*, as a sole food source, producing brood sizes comparable to those observed when the nematode is fed with *E. coli* OP50 (MYLONAKIS et al., 2002). However, the human pathogenic yeast, *C. neoformans*, killed the nematodes. This study also showed that the *C. neoformans* polysaccharide capsule and genes associated with signal transduction pathways, such as GPA1, PKA1, PKR1, and RAS1 as well as laccase production (LAC1) and the α-mating-type gene are associated *C. neoformans* virulence.

Moreover, *C. neoformans* adenine auxotrophs were less virulent in *C. elegans*, similar to the observation in mammals. Both *C. neoformans* and *C. gattii* utilize capsule production as their main virulence attribute as it protects the microorganisms from phagocytosis and has immunomodulatory properties. However, the role of the *C. neoformans* capsule as a virulence factor in *C. elegans* is not as well studied and understood as it is in the murine models. Although heat-killed capsular *C. neoformans* strains were able to kill *C. elegans* much more efficiently than the heat-killed acapsular strains, the killing of the worm by acapsular *C. neoformans* strains showed that the capsule is not necessary for virulence (MYLONAKIS et al., 2002). However, it cannot be ruled out that the acapsular *C. neoformans* strain may still synthesize specific toxic components of the *C. neoformans* capsule that contribute to its virulence.

3.4 *Caenorhabditis elegans* and the *Paracoccidioides* spp.

Paracoccidioides spp. are dimorphic fungi that are causative agents of paracoccidioidomycosis (PMC), which is prevalent in South America (MARTINEZ, 2017). The disease is characterized by progressive granulomatous changes in the nose, sinuses, and skin (BOCCA et al., 2013). In addition, PMC can also affect the gastrointestinal tract, central

nervous system, and skeletal system. The most common spp. capable of causing disease are *P. brasiliensis*, *P. lutzii*, *P. americana*, *P. restripiensis*, and *P. venezuelensis* (TURISSINI et al., 2017). The pathogenesis of *Paracoccidioides* through *in vivo* studies has been characterized using several invertebrate animal models, including *C. elegans*. It was observed in recent studies that *P. brasiliensis* and *P. lutzii* could not be ingested by *C. elegans* due to their large size and irregular shape and, therefore, could not cause infection. However, mere exposure to the fungi elicited an immune response in *C. elegans*, evident from the increased expression of specific antimicrobial peptide genes. The expression levels of antimicrobial peptide genes were also different between the two *Paracoccidioides* spp., as *cnc-4*, *nlpl-27*, and *nlp-31* expression was higher after exposure to *P. brasiliensis* compared to *P. lutzii* (SCORZONI et al., 2018).

Although studying the pathogenesis of *Paracoccidioides* spp. using the *C. elegans* model proved problematic, insights into the differences in the activation of innate immunity between the two spp. were gained. The *G. mellonella* model is a better-suited invertebrate animal model and has been used to study the pathogenesis of *P. brasiliensis* and *P. lutzii* (SCORZONI et al., 2015).

4. *Caenorhabditis elegans* as a virus host

Although *C. elegans* has been widely used as a study organism to assess the pathogenicity of several microbes such as fungi and bacteria, viruses were left out, as there was no known natural viral pathogen of the nematode (GAMMON, 2017). However, with the discovery of the Orsay virus, a natural parasite of *C. elegans*, and also with the development of artificial models of viral infection, studies in the field of virology are increasingly considering this animal model to investigate the various stages of the viral infectious process (GAMMON, 2017; JIANG et al., 2020).

The main models studied are based on RNA viruses resembling the Orsay virus, although DNA virus models have also been developed. The studies mainly seek to evidence the identification and function of factors present in the host, which are necessary for viruses to complete their life cycles since they do not have their metabolism and thus depend on their host cells for the replication of their components (SANDOVAL, 2020).

Identifying cellular factors that facilitate infection is the key to understanding

the viral life cycle and developing drugs with new therapeutic targets (JIANG et al., 2020). With approximately 38% of coding genes orthologous to human genes (GAMMON, 2017), *C. elegans* represents an opportunity to investigate the immune response mediated by genetic pathways and gene functionality in the face of contamination caused by the microbiota (STERKEN et al., 2021).

4.1 RNA virus models in *Caenorhabditis elegans*

One of the first artificial models was developed based on the Flock House virus (FHV), which is an alphanodavirus with high replication capacity in different animal and plant cell types (HUANG; KAMMENGA, 2020). Its genetic material is formed by a single strand of positive-sense RNA (ssRNA) that is divided into two segments: RNA1 and RNA2, and from the RNA1 intermediate, a subgenomic RNA called RNA3 is produced. Even in *C. elegans*, all three types of RNA are produced, and virions cannot form, indicating the lack of a component necessary for the virus assembly (GAMMON, 2017).

A second model was created with the vesicular stomatitis virus, which can infect cells of several vertebrate and invertebrate animals, including *C. elegans*. However, the problem with using this model is due to the way in which viral replication happens. It is necessary to use primary cells derived from embryos for its study. As there are no immortalized *C. elegans* embryonic cells, the use of this model precedes their isolation, an intensive and challenging procedure. This obstacle reflects the low performance of virological studies using *C. elegans* cell cultures (SANDOVAL, 2020).

In both systems, it is observed that a defense mechanism for the infection of the two pathogens is the use of interfering RNA (RNAi), produced by the worm or added to food (HUANG; KAMMENGA, 2020; LU et al., 2005). To overcome the difficulties of *C. elegans* cell culture, the vesicular stomatitis virus was inoculated into adult worms through microinjections. The results indicated that the presence of RNAi restricted the infection to tissues that received the virus and controlled their proliferation in the body of the worms. Moreover, more excellent resistance to viral infection was observed in the offspring whose mother had been subjected to contamination (GAMMON, 2017).

4.1.1 Orsay virus natural model

Orsay virus was isolated from wild nematodes, constituting the first natural viral pathogens of *C. elegans*. They cause infection in intestinal cells causing abnormalities in this area. However, they do not damage the worms' longevity or alter the number of progeny (FRANZ et al., 2014). The transmission occurs only horizontally (GAMMON, 2017).

Studies with this system have already evidenced at least two main components for infection caused by this pathogen. One is the need for the functional *drl-1* gene since the mutant worms had a viral load 10,000 times lower when compared to wild-type worms (SANDOVAL, 2020). Another factor is the presence of HIPR-1. This protein interacts with Huntingtin, which is very important for being a factor in clathrin-mediated endocytosis. The lack of HIPR-1 impairs one of the life cycle stages of the Orsay virus (JIANG et al., 2020).

5. *C. elegans* and other microorganisms

5.1 *Caenorhabditis elegans* as a protozoan host

Diseases caused by protozoa are considered emerging and neglected mainly in developing countries, causing a significant impact on the social and economic spheres and directly affecting public health. Malaria (*Plasmodium spp.*), Chagas' disease (*Trypanosoma cruzi*), and toxoplasmosis (*Toxoplasma spp.*) are some of the diseases that are threatening the lives of one-sixth of the world's population (FLETCHER et al., 2012; GUARNER, 2019). The livestock industry is also affected, and considerable amounts of protozoan diseases are documented, causing herd reductions, and some of them are transmitted to humans (SOARES et al., 2019; TOMLEY; SHIRLEY, 2009). The current drugs used in treating some diseases are becoming resistant or highly effective according to the degree of disease, as is the case with malaria and Chagas disease (FLETCHER et al., 2012). In this context, *C. elegans* would emerge as an excellent model for testing new antimalarial drugs, but studies of new compounds involving *C. elegans* for toxicity tests are scarce. Only one study involving antimalarial drugs was documented until the registration of this article. The effects of new active coumarins

used in the worm were investigated in toxicological tests to select the most appropriate compounds effective against *Trypanosoma cruzi* causing Chagas disease. The results indicated that *C. elegans* was an efficient model for screening these compounds (SOARES et al., 2019).

6. Conclusion

In summary, we demonstrated that *C. elegans* can be used as an appropriate infection model to study the pathogenicity of almost any microorganisms, not only for evaluating the virulence traits of these species but also to screen possible drugs to combat these agents and study mechanisms of the immune response against these yeasts. Future studies should expand the model described here to yield more insights into the pathogenicity of these species.

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Chapter 5

Natural antioxidants against *Caenorhabditis elegans* aging in a nutshell

Priscila Gubert
Elias Almeida Silva Barbosa
Lucas Aleixo Leal Pedroza
Iverson Conrado Bezerra
Marina Meirelles Paes
Gustavo. R. Villas Boas
Michael Aschner

Abstract

Extracts and compounds of natural origin are essential for animal nutrition. Due to advances in studies on oxidative stress and its correlations with aging, it has been noted that several classes of these compounds, such as flavonoids, have antioxidant and anti-aging potential. These properties of natural compounds influence the process of aging, which is a general term for several physiological changes that living beings passes over time although there are intracellular, and molecular pathways that may decelerate or even inhibit such process. In turn, senescence processes states for negative products of aging, which causes degeneration, and increased mortality. Once studies on aging in vertebrate models are time-consuming and costly, the nematode *Caenorhabditis elegans* experimental model has been adopted to characterize the antioxidant efficacy of natural compounds and the molecular pathways responsible for aging. Here we emphasize the variety of plant-based compounds and phytochemicals with antiaging benefits on *C. elegans*.

Key words: *C. elegans*; antioxidants; aging; Oxidative stress; Plant-based compounds.

1. Introduction

The term “aging” comprises a non-specific term used to refer to any physiological change over time while senescence is a deteriorative change that causes increased mortality. Aging derives from multifactorial reasons including significant increase in apoptotic processes correlated with mitochondrial dysfunction (ZHANG et al., 2019)04/12/2022 14:53:00, the main organelle on reactive oxygen species (ROS) production such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2), superoxide anion (O_2^-). Although the damage induced by ROS in cell biomolecules, the enzymes

superoxide dismutase (SOD), catalase (CAT), and other peroxidases constitute mostly the cell's weapons against ROS, once these enzymes can contribute to a less oxidative environment to the cells mitochondrial.

Natural extracts have emerged as efficacious agents in retarding the aging process due to their enormous antioxidant potential as noted both *in vitro* and *in vivo* (ZHENG, 2020). Also, several phytochemicals, secondary metabolites from plants and microbes, acts extending longevity by scavenging ROS, which can be observed among different living organisms, such as: *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Caenorhabditis elegans* (*C. elegans*), mice, rats, and also in human cells (ZHENG, 2020). Although natural extracts and phytochemicals properties have been studied throughout human history, little is known about their impact on aging in alternative experimental models, such as the *C. elegans*.

The current global life expectancy in 2021 is 72.81 years for humans (WORLD LIFE EXPECTANCY, 2022) which is the biggest obstacle in longevity studies. It was with these demands in mind that the nematode *C. elegans* emerged as an outstanding alternative model for longevity studies, since *C. elegans* has an extremely short lifespan (˜ 3 weeks), and a short life-cycle (2.5 days from egg to adulthood, at 25 °C) (APFELD; ALPER, 2018).

Thus, this chapter highlights the effects of plant-based compounds in *C. elegans*' healthspan and lifespan. We also briefly describe the main pathways involved in the aging process and antioxidant response that were investigated under *C. elegans* exposure to plant extracts and isolated phytochemicals.

2. *C. elegans'* Aging mechanisms

C. elegans is a free-living nematode, which conserves the aging process and the antioxidant pathways. In addition, the transparency facilitates the visualization of cells and other biomolecules marked mainly with fluorescent protein constructions (e.g., green fluorescent protein, GFP), such as proteins, organelles and genes (HUNT, 2017). In addition, *C. elegans* has genes with about 60 - 80% homology to humans (BRENNER, 1974), a factor that culminates in highly conserved antioxidant pathways similar to

those known in humans, including the SKN-1 stress response pathway and Insulin / insulin-like growth factor (IIS) (TAYLOR et al., 2020). Consistent with humans, the worms present a range of morphological changes and biomarkers of age-related dysfunction which includes the atrophy of the intestine impaired mitochondrial function (REGMI; ROLLAND; CONRADT, 2014), deficiencies such as mobilitydeficits, impaired stress resistance and reduced immunity (COLLINS ET AL. et al., 2008).

The most common endpoints considered in aging evaluations in *C. elegans* are estimation of the mean and maximum lifespan. However, the extension in both health and lifespan are essential in the clinical domain. Several parameters can be evaluated as indicators of healthspan conditions in worms, such as movement, pharyngeal pumping, and reproduction. This section reviews the most studied pathways in *C. elegans* regarding the aging process, mainly evaluated by plant-based compounds' studies (COLEEN T. MURPHY; PATRICK J. HU, 2018).

In *C. elegans*, aging is associated with oxidative stress and gene expression due to environmental conditions such as temperature changes and diet restriction (DR).

The IIS signaling is one of the most studied pathways in *C. elegans*. The insulin-like peptide ligands (ILPs) bind to the insulin/IGF-1 transmembrane receptor (IGFR), the ortholog DAF-2 in worms. DAF-2/IGFR phosphorylate and activate a conserved phosphoinositide 3-kinase (PI3K)/Akt kinase cascade, which includes the serine/threonine kinases PDK-1, AKT-1, AKT-2, and SGK-1 are activated, culminating in the phosphorylation of a FoxO transcription factor (FOXO), DAF-16, which remains at the cytosol (Figure 1).

The *daf-2* mutants show a decrease in IIS signaling, which leads to DAF-16 (unphosphorylated) translocation to the nucleus, triggering changes in the expression of DAF-16 downregulated genes (TEPPER et al., 2013). As a consequence, the *daf-2* mutants can reach the double of wild-type lifespan and improved aging related- aspects as extended germline maintenance and reproductive span, retain their mobility and healthy appearance longer than wild-type animals

DAF-16 signaling includes increasing gene transcriptions related to metabolism and stress responses (PRINCZ; PELISCH; TAVERNARAKIS, 2020). Consequently, the

reduction of IIS signaling in *C. elegans* improves healthspan inherent features, including axon regeneration, learning process, and resistance to pathogens. Also, suppress neurodegenerative protein aggregation and neuromorphological defects (MACK; HEIMBUCHER; MURPHY, 2018). Once in the nucleus, DAF-16 can activate *skn-1* expression. In *C. elegans,* the *skn-1* (skinhead) gene encodes a transcription factor that is the orthologue of the mammalian NRF2 (Nuclear factor erythroid 2-related factor 2) and activates a detoxification response. *skn-1* promotes resistance to oxidative stress and also increases lifespan. Although, evidence suggests that SKN-1 impact longevity is independent of the oxidative stress response induction (TULLET et al., 2017). Heat shock transcription factor-1 (HSF-1) overexpression has been reported to promote longevity in *C. elegans* in physiological conditions and is related to the IIS and TOR signaling (GUTIERREZ-ZETINA et al., 2021).

UTX-1 modulates the epigenetic and expression status of the IIS pathway genes. UTX-1, the *C. elegans* homolog of mammalian UTX histone demethylase specific for H3K27me2/3, is implicated in the lifespan regulation. Although, paradoxically, both loss and gain of its function trigger improved longevity (KHAN; SINGER; VAUGHAN, 2017).

Another well-investigated mechanism is the DR in *C. elegans,* responsible for increasing the lifespan by at least two independent pathways: the IIS signaling already covered above and Sir2-family deacetylase, SIR-2.1(KAEBERLEIN et al., 2006). Sirtuins are a family of NAD+-dependent deacetylases and highly conserved in *C. elegans* as four Sir2 paralogs, *sir-2.1*, *sir-2.2*, *sir-2.3*, and *sir-2.4* (VISWANATHAN; TISSENBAUM, 2013). *sir-2.1* has been extensively studied because of its homology to the human sirtuin SIRT1. The sirtuin SIR-2.1 uses NAD as a cofactor to mediate the removal of acetyl groups (Ac) from target proteins. Consequently, deacetylated proteins enhance the mitochondrial function that induces positive effects on lifespan and healthspan of worms (VERDIN, 2014). The overexpression of the human SIRT1 gene extends lifespan in *C. elegans*. In addition, the *C. elegans* mitochondrial sirtuins, *sir-2.2*, and *sir-2.3* participate in the lifespan regulation by controlling the oxidative stress response. The *sir-2.2* and *sir-2.3* mutants show increased expression of the mitochondrial SOD, *sod-3,*

and decreased levels of the CAT, *ctl-1* and *ctl-2* (CHANG; MCREYNOLDS; HANNA-ROSE, 2017). As we discuss in the following sections, many plant products can extend *C. elegans* lifespan through SIR-2.1. In addition, the DR can activate the redox enzyme thioredoxin (TRX), conserved as *trx-1* gene, and regulate adult lifespan extension (FIERRO-GONZÁLEZ et al., 2011), whereas *trx-1* mutation causes a decrease in *C. elegans* lifespan (MIRANDA-VIZUETE et al., 2006).

The TOR kinase is activated under nutrient- and energy-sufficient conditions, stimulating growth and inhibiting autophagy. Thus, a reduction in TOR signaling increases the lifespan in *C. elegans* in a DAF-16-dependent manner. The effects on longevity induced by the inhibition of TOR signaling in *C. elegans* are mediated by the transcription factor PHA-4/FoxA, autophagy, and, consequently, a lifespan regulator (UNO; NISHIDA, 2016). *C. elegans* conserves two distinct complexes: TORC1, which impacts lifespan by regulating DAF-16/FOXO and the transcription factor SKN- 1/Nrf, and TORC2 that modulates lifespan mainly through SKN-1/Nrf (SUN; CHEN; WANG, 2017).

On the other hand, the conserved kinase AMPK, encoded by the *aak-2* gene, is an energy sensor implicated in catabolic processes that generate ATP under low-energy conditions. The loss of *aak-2* decreases lifespan and the increased expression of *aak-2* extend lifespan in *C. elegans* (UNO; NISHIDA, 2016).

The Oxidative Theory of Aging is one of the primary explanations for aging development dependent. However, adverse stimuli and ROS inducers in *C. elegans* can trigger the activation of several pathways that contribute to promoting longevity. As the central organelle involved in ROS production, mitochondrial activity is crucial for *C. elegans* lifespan (DENZEL; LAPIERRE; MACK, 2019). Also, mitochondrial dynamics act to stimulate lysosome biogenesis, consequently benefiting longevity in the worm (LIU et al., 2020).

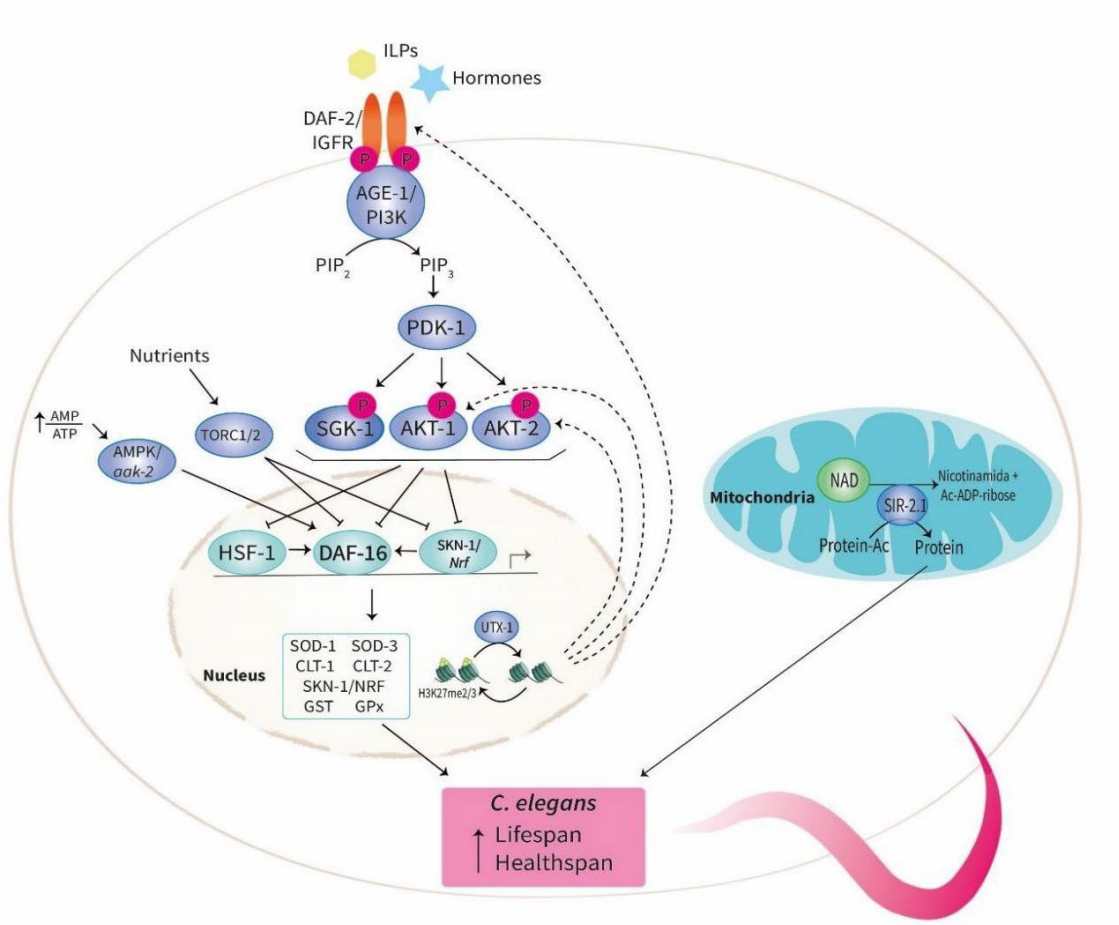

Figure 1: Crucial Aging-related machinery to Plant derived compounds' effect. Abbreviations: AMPK, AMP-activated protein kinase; CAT, catalase (orthologous, CLT-1 and CLT-2); ILPs, insulin-like peptides; HSF-1, heat shock transcription factor-1; GPx, glutathione peroxidase; GST, glutathione-S-transferase NAD, Nicotinamide adenine dinucleotide; PDK-1, Pyruvate Dehydrogenase Kinase 1; PI3K, phosphoinositide 3-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; SGK, Serine/threonine-protein kinases; SOD, superoxide dismutase; TOR, target of rapamycin (orthologous, TORC1 and TORC2).

As a by-product of mitochondrial activity, the O_2^- has been posited to be a potential contributor to aging. SOD converts O_2^- into H_2O_2 and O_2, whereas CAT and peroxidases (e.g., glutathione peroxidase (GPx)) convert H_2O_2 into water.

C. elegans expresses two mitochondrial MnSODs, encoded by *sod-2* and *sod-3*; two cytosolic Cu/ZnSODs, encoded by *sod-1* and *sod-5*; and two predictedextracellular Cu/ZnSOD isoforms, both encoded by *sod-4*. Among them, *sod-1* appears as more implicated in aging once its overexpression caused a slight increase in lifespan, and the loss reduced the lifespan (GEMS; DOONAN, 2009). *C. elegans*expresses three CAT enzymes: *ctl-3*, *ctl-2*, and *ctl-1* arranged in a tandem array.

Peroxisomal CTL-2 is responsible for ~80% of the total CAT enzymatic activity and the impacts on lifespan, which is reduced in *ctl-2* mutants (BACK; BRAECKMAN; MATTHIJSSENS, 2012). Nevertheless, GPx plays a role in reducing H2O2 by using the endogenous antioxidant glutathione (GSH) as an electron donor. GPx isoforms in *C.elegans* did not present the selenocysteine at the active site and were responsible forthe catalytic activity that may suggest its mechanism differs from that in mammals (FERGUSON; BRIDGE, 2019). The antioxidant response includes detoxification enzymes, including glutathione-S-transferases (GSTs), responsible for removing oxidative stress products such as 4-hydroxynonenal (HNE). *gst-10* mutation and overexpression are related to decreased and increased *C. elegans* lifespan, respectively (AYYADEVARA et al., 2005).

3. Plant-based Compounds and Aging

Plants and endophytes, microbes that inhabit plant-tissues can practice commensalism or mutualism, had adapted to synthetize millions of secondary compounds, which provide them new chemical weapons to combat endogenous and exogenous stressors (WANG et al., 2019). These chemical weapons are the phytochemicals, that constitute an enormous variety of chemical groups, such as: phenolic compounds, for example, Coumarin, Ligin, and Flavonoids; Terpenes, which include Carotenoids, Monoterpenes, Saponins, and others; Organosulfur, such as Allylic sulfur compounds, glucosinolates, indole; Betalains; Polysulfides, and others. Functionally, they act as antioxidants, antimicrobials, absorbents of UV-light, and play a role in other protective systems in plants that increase the longevity of plants.

According to that, we highlight below some phytochemical classes that combines its antioxidant properties to aging impairment phenotypes in the *C. elegans* model. The main mechanisms on plant extract and phytochemical effects in *C. elegans*' lifespan and healthspan were combined in Table 1 and discussed in section 4 and 5.

4. Plant Extracts

4.1 Citrus

Citrus, commonly derived from the *Rutaceae* family plant products, are mostly

known as orange, lemon, tangerine, grapefruit, and others. Citrus compounds are highly associated with antioxidant, anti-inflammatory, modulation of lipid metabolism, reduction of diabetes effects, anti-senescence properties (MAHMOUD et al., 2019). These compounds can be grouped into classes: Flavonoids (noticeably by naringin, hesperidin, naringenin), Phenolic acids; Coumarins, and Terpenoids, such as Limonoids (limonin 17-β-D-glucoside (LG), obacunone 17-β-D-glucoside 171 body (OG), millington acid 17-β-D-glucoside (NAG) and deacetylation millington 172 acid 17-β-D-glucoside (DNAG)), and Carotenoids.

When applied to myocardial H9c2 cells and old rat heart, showed that the cells treated with bergamot juice increased the expression of anti-aging and antioxidant responses associated genes: SIRT1, NRF2, and FOXO3 (DA POZZO et al., 2018). In addition, naringenin, a citrus flavanone, showed efficacy against ROS induction and senescence in myocardial H9c2 cells by activating metabolic pathways in mitochondria. In *Drosophila melanogaster,* both hesperidin and limonene increased healthspan and lifespan (FERNÁNDEZ-BEDMAR et al., 2011).

In *C. elegans*, the orange extract can increase the lifespan of *C. elegans* N2 Bristol wild-type and SOD and CAT enzyme activities while reducing intracellular ROS levels. In addition, the orange extract enhanced thermotolerance and resistance to UV- B radiation. These results combined with the upregulation of gene expression, such as *daf-16*; genes on MAPK pathway (*sek-1* and *skn-1*), and antioxidant related genes, such as *sod-3* and *gst-4*, demonstrate that the orange extract might reinforce both survivaland lifespan (WANG et al., 2020).

Table 1: Plant extracts, Phytochemical, and genetic mechanisms related to lifespan and healthspanbenefits in *C. elegans.*

Plant extract	Influenced genes	Phytochemicals	Influenced genes
Citrus	The sek-1, skn-1, sod-3, and gst-4 genes are related to the survival and lifespan enhancing (WANG et al., 2020).	**Procyanidins**	Apple procyanidins (epicatechins and catechins) prolonged the mean lifespan by acting on the *sir-2.1* gene (SUNAGAWA et al., 2011).

Blueberry	Extended *C. elegans*lifespan by 44% (WILSON et al., 2006). Affected the mRNA expression levels of HSP 12.6, 16.1, 16.49 and 70. Apple + blueberry extract generated resistance to stress via the SKN-1 pathway (SONG et al., 2020).	**Carnosol**	Increased the activity of SOD, CAT, and GSH-Px, also theexpression of *sod-5*, *sod-3*, *hsp-1*, *hsp-16.1*, and *hsp-16.2*. Carnosol prolonged the lifespan of *C. elegans* and promoted nuclear translocation of DAF-16 (LIN et al., 2019).
Apple	Promoted an increase in the mean lifespan (VAYNDORF; LEE; LIU, 2013).	**Quercetin**	Antioxidant activity involved with *daf-2*, *age-1*, *nsy-1*, *sek-1*, *pmk-1*, *skn*-1, and *hsf-1* (AYUDA-DURÁN etal., 2019).
Senna Singueana	Reduced endogenous ROS by 47%, increased the DAF-16 transcription factor in the nuclear region (76.19%) (SOBEH et al., 2017).	**Resveratrol**	Regulated positively *sirt-1* and *sir-2.1*. Down-regulates genes responsible for the lipogenesis and biosynthesis of fatty acids, such as F49E12.10, F49E12.9 or *drd-1*, *fat-7*, *acdh-1*, *acdh-2*, and *cpt-4* (ARANAZ et al., 2020).
Calycophyllum spruceanum	Improved the antioxidant activity and extended the average lifespan by 77% (PEIXOTO et al., 2018) and acted through DAF-16.	**Tomatidine**	Protected against the loss of age-dependent muscle function by activating the Nrf2/SKN-1-DCT-1 pathway. In addition, it is involved in the metabolism of ROS (FANG et al., 2017).

Cleistocalyx nervosum	Positively regulated *egl-8* and *egl-30* genes improving lifespan. Aging-related genes, such as *col-19*, *dgk-1* and *goa-1*, were down-regulated. Positive regulation on *daf-16* and negative on *daf-2*, *age-1*, and *utx-1* (PRASANTH et al., 2019).	**6-shogaol and 6-gingerol**	Reduced ROS and increased the SOD-3 and CAT activities,and GFP expression of SOD-3 and HSP-16.2. Decreased the expression of lipofuscin, improving healthspan in elderly worms (LEE et al., 2018a).
Cassia fistula	Induced the nuclear localization of DAF-16 and decreased *gst-4* expression (THABIT et al., 2018).	**Astaxanthin**	Increased the expression of daf-16 target genes such as sod-3, sod-5, ctl-1, and ctl-2 (LASHMANOVA et al., 2015).
Peppermint	Decreased ROS levels and increases the lifespan (WU et al., 2019).	**Caffeic acid**	It negatively regulated *hsp-3*, *hsp-16.1*, *hsp-16.41*, *hsp-17* and *hsp-70* gene expression. Increased HSP-12.6 expression (COLONNELLO et al., 2020; GUTIERREZ-ZETINA et al., 2021).

4.2 Blueberry

Some polyphenols-based plant extracts, such as blueberry (*Vaccinium myrtillus*, *Vaccinium angustifolium*, among others) extract, have also been shown to induce anti-aging mechanisms in *C. elegans* (SONG et al., 2020). Blueberry extract (BE) increased *C. elegans* lifespan in days up to 44%, both in a temperature and dose-dependent way. Moreover, BE extract reduced intestinal levels of lipofuscin concentration, a pigment that accumulates in an age-dependent manner, and increased by 21% *C. elegans* lifespan when exposed to paraquat, an herbicide that induce ROS in animals. BE also diminished lipid peroxidation indicator, 4-Hydroxynonenal. However, it is reported that BE may interfere with worm fertility (WANG et al., 2018).

The BE, especially the proanthocyanidin-riched fraction, extended *C. elegans* lifespan by interfering in aging pathways related to thermotolerance. The BE extract

caused a lifespan improvement of 28% in wild-type worms (WILSON et al., 2006).

However, the BE did not show any effects under oxidative stress conditionsupon paraquat or H_2O_2 treatments. BE affected the mRNA expression levels of *hsp 12.6, 16.1*, *16.49* and *70.* The heat shock protein (HSP) are a class of proteins overexpressedin old-lived animals, in which the BE could maintain the expression at basal levels, even in adult worms (L4 larval phase) (WILSON et al., 2006).

In addition, there are synergistic effects of apple extract (APE) and BE that increase *C. elegans* lifespan and ameliorate the resistance against oxidative stress. It was reported that the combination of APE and BE increased wild-type worm lifespan up to 31.4% at control or stress (paraquat) conditions. In addition, the combination had upregulated the expression of enzymes that combats ROS formation, such as SOD, CAT, and GSH, while reduced malondialdehyde (MDA), a marker pf lipid peroxidation (TSIKAS, 2017), was downregulated compared to the control group. TheAPE + BE combination acts via the SKN-1 transcription factor, which is thought to improve oxidative stress resistance by downstream gene expression in the worm (SONG et al., 2020). According to (VAYNDORF; LEE; LIU, 2013), the whole apple extract increased *C. elegans* lifespan.

4.3 Agrimonia procera

Agrimonia procera (A. procera) extract is rich in polyphenols and has beneficial health effects as an antioxidant outcome of its phytochemicals such as agrimoniin, and flavonoids generally defined as glycosides luteolin and apigenin (GRÄBER et al., 2018).

After treatment with *A. procera*, a significant increase in worms' survival that underwent thermal stress (37°C) and reduction in ROS levels were observed. In the lethal thermal stress tests the average lifespan of the stressed worms treated with *A. procera* was extended by up to 7,5% compared to the untreated group (SAIER et al., 2018). Although any changes in DAF-16 transcription factor translocation wereobserved after *A. procera* treatment, the extract lost its protective capacity when *daf-16* mutants were exposed to lethal heat stress and paraquat (SAIER et al., 2018).

4.4 Calycophyllum spruceanum

Calycophyllum spruceanum, commonly known as *pau-mulateiro* in Brazil, is an amazonian plant with a paramount role in traditional Indian medicine. Traditionally, *C. spruceanum* is generally applied to dermal and skin problems, varying from mycosis to wounds and cuts. However, no scientific evidence shows that *C. spruceanum* corroborates such a role (DA SILVA et al., 2018). Nonetheless, there is evidence of beneficial properties, such as antioxidant and anti-inflammatory. The extract of *C. spruceanum* is rich in phytochemicals, as seco-iridoids, flavonoids, anthocyanins, and coumarins (DE VARGAS et al., 2016).

In *C. elegans*, an extract from the stem bark portion of *C. spruceanum* had shown antioxidant functions and extended the average lifespan of treated worms (PEIXOTO et al., 2018). The phytochemical characterization demonstrated that it was mainly composed of gardenoside, cyanin, 5-hydroxymorin, taxifolin, and 5-hydroxy-6-methoxycoumarin-glucoside. Furthermore, the antioxidant activity of *C. spruceanum* extract was analyzed via the exposition of wild-type worms previously exposed to juglone, a toxic compound derived from walnuts that in high concentrations, caused oxidative stress by producing ROS. *C. spruceanum* extract could increase worm lifespan up to 77% compared to the control group. Also, when applied to the *C. elegans* female BA17 [*fem-1*(hc17)] strain, the Amazonian extract delayed the aging process and extended the lifespan up to 16%, if compared to the control group. However, the same results were not approachable in the *daf-2* mutant strain when *C. spruceanum* extract had not significantly extended the worms' lifespan. Additionally, *C. spruceanum* extract had not changed *C. elegans* brood size, body length, and DR. On the other hand, *C. spruceanum* extract could influence the pharyngeal pumping rate by decreasing it over time, which might corroborate its anti-aging properties. Together, results point that *C. spruceanum* extract is acting through the canonical aging-related transcription factor, DAF-16.

4.5 Cleistocalyx nervosum

Cleistocalyx nervosum var. paniala (*C. nervosum*) is an extract from an Indian fruit widely grown in Thailand. It belongs to the *Myrtaceae* family, a group that is already

known for the medicinal effects such as anti-carcinogenic activities due to some molecules rich in phenolic and anthocyanin groups, which are, in turn, non- glycosylated water-soluble molecules belonging to the group of flavonoids (CHARIYAKORNKUL et al., 2021).

In the study by Prasanth and coworkers (PRASANTH et al., 2019), two extracts of *C. nervosum,* CMK-P and LMK-P, were collected from the Thai cities Chiang Mai and Lampang, respectively. Both compounds prolonged the mean and maximum lifespan in *C. elegans*.

It was also noted that both compounds failed to decrease the rate of pharyngeal pumping while reducing the lipofuscin levels, thus indicating an improvement in the worms' healthspan. CMK-P and LMK-P extracts induced a positive regulation of responsible genes for improving the animals' health lifespan, such as *egl-8* and *egl-30*. In contrast, genes related to advancing age, such as *col-19, dgk-1* and *goa-1*, were negatively regulated (PRASANTH et al., 2019). Besides, both extracts caused a positive regulation of *daf-16* and downregulation of *daf-2*, *age-1*, and *utx-1*, indicating a dependence on the transcription factor *daf-16* activity.

C. nervosum also played an essential role in decreasing the levels of oxidative stress observed in worms exposed to UV-A radiation by the positive regulation of *skn-1* and *sir-2.1* genes responsible for activating antioxidant pathways and, consequently, by reducing ROS (PRASANTH et al., 2019).

4.6 Cassia fistula

Cassia fistula Linn., which is part of the *Leguminosae* family, diffuses over the globe with specimens in countries such as India, Brazil, South Africa, China, Mexico, and others.

The antioxidant potential of *Cassia f.* extract had been analyzed in *C. elegans* (THABIT et al., 2018)The hydroalcoholic extract was composed mainly of quinic acid derivatives, and several glycosides.

In terms of biological effects, the *C. fistula* extract in *C. elegans* was able to increase survival rates of worms exposed to juglone compared to the control group. Also, when compared to the same control group, the extract could decrease up to 73%

the intracellular levels of ROS. Furthermore, *C. fistula* induced the nuclear localization of DAF-16 accompanied with the following results: decrease of *gst*-4 gene expression in gpIs1 [*hsp-16*.2$_p$::GFP] mutant worms that contains the presence of a GFP coupled with HSP-16.2, showed a lower expression of HSP-16.2, induced by juglone when compared to solvent control + juglone treated group, and a higher expression of the SOD-3::GFP compared to the negative control. Finally, the *C. fistula* extract benefits appears to be dose-dependent, since worms treated with the highest concentration showed a 67% increase in fluorescence intensity of SOD-3, which increased the lifespan and stress response (THABIT et al., 2018).

4.7 Licorice

Licorice (*Glycyrrhiza glabra*) is widely used in the production of candies due to its sweet taste and commonly used in Chinese medicine due to its anti-allergic and anti-inflammatory activities. Such effects are attributed to the high concentration of the triterpenoid saponin phytochemicals (glycyrrhizic and glycyrrhizic acid) (WANG et al., 2013).

In *C. elegans*, its beneficial effects on anti-aging processes were notable, since, in worms treated with Licorice, a lifespan and resistance to stress was observed upon treatments with juglone, paraquat, and thermal stress (REIGADA et al., 2020; RUAN et al., 2016). In addition, it caused improvements in healthspan, such as brood size and locomotion in worms exposed to the toxic compound graphene oxide (ZHAO et al., 2016). Licorice affected the Aβ aggregation and its cytotoxic effects. Licorice could reduce not only Aβ-dependent paralysis and reduce protein aggregation (LINK et al.,2015).

Concerning gene expression regulation, it was noted that *pdk-1*, *daf-18*, and *daf- 16* were up-regulated, to the same extent that *daf-2, age-1*, and *sgk-1* were down- regulated. Mutant *daf-16* worms had the beneficial effects of lifespan suppressed in addition to an increase in ROS (RUAN et al., 2016). Also, Licorice induced the translocation of the DAF-16 transcription factor to the nuclear region, reduced the expression of HSP-16.2 and increased the expression of SKN-1 proteins (LINK; WINK, 2019).

miRNAs were evaluated after Licorice treatment in worms exposed to the

graphene oxide stressor. *mir-4805* and *mir-1820*, responsible for *sod-1* regulation, were up-regulated, as well as *mir-360* and *mir-246* (*sod-2*), *mir-392* (*clk-1*), and *mir-4810* (*gas-1*) (ZHAO et al., 2016).

5. Phytochemicals

5.1. Apple procyanidins

The (-) epicatechins and (+) catechins are examples of 2 procyanidins common in apples. In humans, the (-) epicatechins and (+) catechins affected cardiovascular diseases by contributing the vascular function improvement (RAMIREZ-SANCHEZ et al., 2018), neurodegenerative disorders (WEINREB et al., 2009), and others by scavenging ROS radicals in addition to chelating metal properties (BERNATONIENE; KOPUSTINSKIENE, 2018). In *C. elegans*, apple procyanidins prolong the mean lifespan of both wild-type and *fem-1* strains by 12.1% and 8.4%, respectively (SUNAGAWA et al., 2011). Moreover, when the test was carried out with apple polyphenols, an increase of 12.0 % and 5.3% was observed in wild-type and *fem-1* worms' mean lifespan compared to the control treatment. It was further noted that the most critical fraction contributing to the lifespan extension was the procyanidins, where the epicatechins and catechins are found. Despite those results, another study has shown that neither the purified monomeric fraction of apple at 35 ng mL^{-1} polyphenols nor 65 ng mL^{-1} epicatechin promoted no change in *C. elegans fem-1* and N2 strains lifespan, respectively. In addition, this study revealed that apple procyanidins in worms may act through *sir-2.1* (SUNAGAWA et al., 2011).

5.2 Compounds derived from peppermint

Peppermint is a specie of mentha with an abundance of phenolic groups (PAVLIĆ et al., 2021) obtained from steam distillation already widely targeted by the pharmaceutical and cosmetics industry due to its antioxidant potential and has even been used to treat several symptoms of patients in the chemotherapy process, such as sleep quality and nausea (HAMZEH; SAFARI-FARAMANI; KHATONY, 2020).

In *C. elegans*, peppermint essential oil was used against the stress conditions exposure induced by H_2O_2. It was observed a decrease in the ROS levels and an increase

in worms' lifespan. These effects were compared with other mentha species, where peppermint demonstrated antioxidant and pro-survival effects at concentrations of 5 μg mL^{-1} compared to the others (100 μg mL^{-1}) (WU et al., 2019).

Among the phytochemical analyzed in peppermint essential oil, a substantial fraction of menthol (38.45%), menthol (21.8%), 1.8-cineole (5.62%), and neo-menthol (4.19%) was noted corroborating other studies where the same compounds have been shown to possess antioxidant activity (MCKAY; BLUMBERG, 2006).

5.3 Carnosol

Carnosol is a polyphenol found in rosemary, lavender, and sage plants. In addition to its antimicrobial, anti-inflammatory, and anticancer effects, studies carried out with rats demonstrated that carnosol is more promising than vitamin C and E concerning the elimination of hydroxyl groups, protection of DNA, reduction of lipid peroxidation, and increase in activity of antioxidant liver enzymes (SHI et al., 2020).

Carnosol reduced the endogenous ROS levels in *C. elegans* exposed to mitochondrial oxidative stress inducers, such as paraquat, and reduced the ROS levels in worms under normal conditions up to 76% (LIN et al., 2019). There was also an increase in the activity of antioxidant enzymes SOD, CAT, and GSH-Px by 42%, 18%, and 87%, respectively, and a significant reduction in lipid peroxidation (21%).

The experiments with *C. elegans* also showed that carnosol could increase the survival curve significantly and positively increased in 19% the average lifespan and an increase of 26% in the maximum lifetime. Worms treated with carnosol extract also showed a significant improvement in healthspan since it was shown to inhibit the age-related decline of body bends mainly in the early and middle stages, in addition to playing a pivotal role in reducing intestinal lipofuscin. However, carnosol was not able to reduce fat accumulation (LIN et al., 2019).

Proteinopathies are often the cause of a massive increase in oxidative stress, especially in neurons (SIDOROVA; DOMANSKYI, 2020). Such proteins as β-amyloid (Aβ), TDP-43, and PolyQ cause mitochondrial damage and increase endogenous ROS levels. Such factors are directly linked to the progression of neurodegenerative

diseases and a drastic reduction in expectation and health-related quality of life (REDDY; OLIVER, 2019).

However, carnosol prolonged *C. elegans* lifespan in transgenic models for Alzheimer's and Huntington's disease. In worms with paralysis induced by Aβ and PolyQ, after treatment with carnosol, a reduction of 21% and 14%, respectively, was noted in their paralysis rates. The genes *sod-3* and *sod-5* were evaluated after carnosol extract exposure, and a significant increase in their expressions was noted, while *clt-1* and *ctl-2* showed no significant changes. Carnosol was also responsible for increasing the relative levels of *hsp-1*, *hsp-16.1* and *hsp-16.2* mRNA, a factor that could justify not only greater longevity, but also the reduction of protein aggregation (LIN et al., 2019).

DAF-16 transcription factor was also evaluated, indicating greater nuclear translocation (60% ± 2%) in the carnosol treated group, while in the intermediate and cytosolic region it was (37% ± 3%) and (3% ± 3%), respectively (LIN et al., 2019).

To verify whether DAF-16 nuclear translocation after carnosol treatment hadany relationship with the IIS pathway, analyses of *daf-2* and *daf-16* gene expressionwere performed. However, there was no change in gene expression. Also, carnosol prolonged the lifespan of *C. elegans* even *daf-16* mutants, thus suggesting the extract ability to induce nuclear translocation of DAF-16, while does not act on its transcriptional activity (LIN et al., 2019).

5.4 Flavonoids

Flavonoids are a large group of polyphenolic compounds widely present in the human diet because they originate in plants and other natural extracts (ERDMAN et al., 2007). Currently, these molecules have received great attention from the pharmaceutical industry due to their ability to mimic the process of caloric restriction in living organisms (ALUGOJU; PERIYASAMY; DYAVAIAH, 2020), as, in several studies, it has been reported that caloric restriction increases ROS production playing key roles in signaling pathways that regulate aging (WEGMAN et al., 2015).

In several living organisms, such as *C. elegans*, kinase proteins, such as phosphatidylinositide 3-kinase and TOR-like signaling proteins, which, when

activated, are responsible for carrying out anti-mitotic processes and for the production of reactive species, mechanisms that are known to accelerate aging (UNO; NISHIDA, 2016).

5.5 Resveratrol

Resveratrol is a stilbene found in grapes and red wine and is already being used by the pharmaceutical industry as a supplement (MOVAHED et al., 2020). Resveratrol has long been proposed as a promising phytochemical in the treatment of pathologies such as metabolic syndromes (BREMER, 2014), neurodegenerative diseases (MOUSSA et al., 2017) and even as an anti-viral capable of reducing the damage caused by SARS-CoV-2 (YANG et al., 2021).

In *C. elegans*, resveratrol acted as an anti-aging agent improveming behavioral phenotypes in worms exposed to stress or upo aging. The improved performance secondary to reservatrol treatment were mediated via autophagic process dependent on SIRT-1 (MORSELLI et al., 2010). Although the AMPK pathway is fundamental in the performance of the antioxidant activities of resveratrol, daf-16 was not intrinsic to the functions of this polyphenol (YOON et al., 2019).

Resveratrol negatively regulates genes responsible for proteins involved in the lipogenesis and biosynthesis of fatty acids such as *F49E12.10*, *F49E12.9* or *drd-1*, *fat-7*, *acdh-1*, *acdh-2* and *cpt-4*, reducing the lipofuscin pigments. In addition, genes related to fatty acid hydrolysis and peroxisomal β-oxidation were up-regulated after treatment with Resveratrol such as *Y51H4A.5*, *F09C8.1* (human PLB1 orthologous) and *F25E2.3* (human *ACOT8* orthologous) (ARANAZ et al., 2020).

5.6 Quercetin

Widely present in fruits such as apples and cherries and other vegetables such as capers and onions, quercetin is a flavonoid that contains phenolic acids such as 3,4-dihydroxyphenylacetic acid and 4-hydroxylphenylacetic, able to promote longevity *in vivo* and *in vitro* models in addition to promoting antioxidant, anti-inflammatory, neuroprotective, and anticarcinogenic activities (LIN et al., 2019).

In *C. elegans,* the increase in lifespan combined to the improvements in the

healthspan of elderly worms after treatment with quercetin is mainly due to its antioxidant activity. It depends on several pathways and genes that respond to different forms of stress, such as *daf -2*, *age-1*, *nsy-1*, *sek-1*, *pmk-1*, *skn-1*, and *hsf-1* (AYUDA-DURÁN et al., 2019; SAUL et al., 2008). Such factors are a strong indicator that quercetin works in combination with interconnected pathways, such as TGF-β, p38 MAPK, and the DAF-12 receptor (2012; SUGAWARA; SAKAMOTO, 2020).

However, when treated with quercetin, it was possible to observe a lifespan increasing and the improvement of factors compatible with healthspan. Molecular evaluations evidenced the activation of the autophagic pathway against the induction of cytotoxic aggregates and reduction of germ cell DNA damage after exposure to UV-B (CIVELEK et al., 2020; LI et al., 2020).

5.7 *Zingiber officinale* phytochemicals

Studies carried out with two isolated compounds (6-shogaol and 6-gingerol) of *Zingiber officinale* (*Z. officinale*), a herbal medicine used in the treatment of gastrointestinal diseases, aging, and metabolic syndromes (MOHD SAHARDI; MAKPOL, 2019) was performed on *C. elegans* to assess its antioxidant and anti-aging effects. In both cases, the increase in endogenous ROS was induced by exposure to osmotic stress (juglone) and heat shock, while the enzymatic expression in response to thermal shock demanded worm maintenance at 36 °C for 25 hours. The isolated extracts 6-shogaol and 6-gingerol were effective in increasing the lifespan of worms,both in N2 wild-type worms that have not undergone previous stress treatment as well as in treated worms (LEE et al., 2018b).

Both SOD-3 and CAT activities, when analyzed by spectrophotometric measurement, as well as the expression of *sod-3* and *hsp-16.2* were increased in worms treated with both *Z. officinale* compounds. the decay of age-dependent locomotion was improved after treatment with *Z. officinale* isolates as well as a reduction in lipofuscin expression, thus demonstrating that these phytochemicals can improve healthspan in elderly worms (LEE et al., 2018b).

5.8 Curcumin

A metabolite derived from the turmeric spice, curcumin, is currently related to anti-aging, antioxidant, anti-inflammatory, neuroprotective, hepatoprotective, and other properties that have been extensively reviewed by ZIA *et al.*, 2021. The turmeric spice, *Curcuma longa*, belongs to the Zingiberaceae family and is mainly composed of a class of natural polyphenols, the curcuminoids. Curcumin, or diferuloylmethane, demethoxycurcumin, and bisdemethoxycurcumin are the most common polyphenols in the turmeric extract curcumin the most present.

When evaluating the physiological effects of curcumin in *C. elegans,* it was observed that rather than through its antimicrobial effects, curcumin prolonged worms' lifespan via its antioxidant properties, increasing the average lifespan from 8.4 ± 0.3 days up to 11.7 ± 0.4 days (LIAO et al., 2011). In addition, the analysis of body length and pharyngeal pumping rate showed that worms treated with curcumin were shorter and had less pumping rate than non-treated worms. However, curcumin did not affect *C. elegans* reproduction and fertility, commonly associated with increased lifespan derived from stress resistance. Decreased body length and pharyngeal pumping rate mechanisms in *C. elegans* are related to stress resistance and DR, extending worms lifespan. Furthermore, the anti-aging effects promoted by curcumin were related to a *daf-16* independent action, once *daf-16* mutants had increased lifespan when exposed to curcumin (20 µM). In addition, in *mev-1* mutants, a subunit of complex II of mitochondrial electron transport chain, which is highly sensitive to ROS, curcumin had enhanced worms survival rate, thus directly affecting mitochondria to combat oxidative stress (LIAO et al., 2011).

5.9 Tomatidine

Tomatidine is a metabolite of tomatine, the main compound of green tomatoes (*Lycopersicon pimpinellifolium*), with beneficial activities in other organisms such as anti-inflammatory, anti-tumorigenic, and other activities such as increased content of mitochondrial DNA and improving muscles in mice with atrophy (HSIEH et al., 2020).

In *C. elegans*, tomatidine increased the lifespan, although toxic effects were

observed in animals exposed to high concentrations (> 50 µM tomatidine). In the behavioral assays, tomatidine improved the feeding behavior and swimming performance at older ages (FANG et al., 2017).

Though showing significant improvement in the actin filaments, tomatidine treatment resulted in the protection from the loss of age-dependent muscle function by activating the Nrf2/SKN-1 pathway, positively regulating mitophagy (FANG et al., 2017). Such findings are consistent with studies that demonstrate an increase in lysosomal and autophagic activity as protective mechanisms against neuronal damage in *C. elegans* (CORDEIRO et al., 2020).

Tomatidine also played a fundamental role in mitochondrial function andROS's metabolism due to increased oxidative phosphorylation of free amino acids contributing to more significant catabolism and an eventual increase the amount of well-located mitochondria in myofilaments (FANG et al., 2017).

5.10 Xanthone

Phytochemicals belonging to the class of xanthones are organic compounds derived from plants such as *Clusiaceae* and *Garcinia mangostana* with several beneficial effects already reported in the literature, such as anti-inflammatory, anti-carcinogenic and anti-diabetic activities (IBRAHIM et al., 2019). Although some studies also indicate that these compounds have anti-aging properties (LI et al., 2019), the mechanisms by which these molecules use for this purpose is still not well understood.

Tang and coworkers (TANG et al., 2019) evaluated thirty molecules belonging to the class of xanthones for their antioxidant and anti-aging potential in *C. elegans*. Analysis of the structure and activity of these compounds indicated that the number and position of substituent groups on benzene rings of xanthones are significant for maintaining their antioxidant activity. Generally, xanthones with higher oxygenation perform better than those that do not.

In the lifespan tests, worms treated with XD-2, 3, 4, 5, 9, 14, 15, 16, 17, 21, 22, 23, 24, 25, 26, 27, 29 xanthones presented increased lifespan from 21.0 ± 1.0 to 27.6 ± 1.6 days, compared to the control groups. Also, XD-2 improved the resistance to paraquat-

induced stress by increasing the lifespan from 13.1 ± 0.93 days to 19.1 ± 1.65 days. However, it was observed that even XD-2 could not increase the lifespan in *daf-16* and *pmk-1* mutants, showing that XD-2 can modulate the anti-aging effects through these two genes (TANG et al., 2019).

During stress tests, paraquat inhibited *daf-16*, *sir-2.1*, *akt-1,* and *age-1*, while increasing *skn-1* and *pmk-1* gene expression. However, after XD-2 treatment, the effects on *daf-16*, *sir-2.1*, *akt-1*, and *age-1* expression were recovered to control levels. All these data indicate that XD-2 acts in a protective way modulating the resistance of stress response (TANG et al., 2019).

Following the data cited above, the xanthone isoxanthohumol also showed antioxidant and pro-longevity activity (increased lifespan) in a DAF-16-dependent way since its protective effects were lost in *daf-16* mutants. In addition, isoxanthohumol has been shown to increase the nuclear sub-localization of DAF-16 (BÜCHTER et al., 2016).

5.11 Astaxanthin and Fucoxanthin

Astaxanthin (AST) is part of the xanthophylls class of carotenoids and can be found in various structures in different organisms, such as algae, yeast, crustaceous, and even in bird feathers, which is the case of flamingos. AST acts as an inducer of antioxidant pathways, collaborating with the neutralization of free radicals and inhibiting lipid peroxidation. It contributes to improving the immune system during inflammation, blocking the nuclear factor NF-κβ, and modulating the expression of the inflammatory cytokines and has a particular neuroprotective function. Once astaxanthin traverses the blood-brain barrier it induces its antioxidant, anti-inflammatory, and other properties in the brain (SZTRETYE et al., 2019).

In *C. elegans*, AST extended lifespan by 16% to 30% both in the wild type and *age-1* strain, after continuous treatment with the phytochemical (YAZAKI et al., 2011). Tests were also carried out on *C. elegans* with carotenoids similar to AST, such as fucoxanthin, in which the individual molecule also increased the lifespan of the worms and demonstrated antioxidant activity in *D. melanogaster* (LASHMANOVA et al.,

2015). On the other hand, AST did not affect *daf-16* null allele mutants lifespan, while the extension promoted by AST in other strains was related to a mRNA expression improvement in DAF-16 target genes via ISS pathway, such as *sod-3*, *sod-5*, *ctl-1*, and *ctl-2* (LASHMANOVA et al., 2015). These genes encode SOD and CAT enzymes in different cellular compartments. A study performed by (YAZAKI et al., 2011), has elucidated that AST in worms, decreased mitochondrial ROS levels and then indirectly protecting intracellular organelles from lipid peroxidation and other oxidative damage.

It is noteworthy that the ability of AST to scavenge ROS derives from its hydroxyl and ketone groups at the extremes of the molecule. However, geometric variances of the molecule structure are related to the third carbon of the ionone rings, resulting in the following stereoisomers: a S enantiomer; its opposite, the R enantiomer; and a *meso* (M) form.

Given the above, a recent study aimed to evaluate how such stereoisomers could have different biological properties in the nematode *C. elegans* (LIU et al., 2016). AST fractions could increase the worm's lifespan, a 19% increase in the 3rd day and 110% increase in the 5th day in comparison to the control group, it was not observed any significant difference between the stereoisomers. Secondly, in an intracellular ROS production test, worms treated with the S stereoisomer showed the highest level of ROS decrease, 40.12%, while the R and M isoform presented 30.05% and 22.04% rate of decrease compared to the control group. Although AST stereoisomers did not show any statistically significant difference for SOD and CAT enzymes activities – the Astaxanthin stereoisomers led to increased SOD activities varying between 44 - 51%, and CAT, 77 - 90%, in an experiment with a transgenic strain containing a GFP reporter in the *sod-3* gene. The S stereoisomer showed the biggest effect in GFP expression of SOD from the 1st until the 3rd day. Furthermore, R and S AST enantiomers were related to several gene expression modulations, especially those involved with the IIS signaling; DR; oxidoreductase mechanism, and other systems related to stress.

5.12 Caffeic acid

Caffeic acid phenethyl ester (CAPE), a natural phenolic compound derived

from honeybee hive propolis. This polyphenol has anti-inflammatory effects already reported in macrophage cell cultures, antioxidant effects in the microglia of the hippocampus of mice (SCHRÖTER et al., 2019).

In *C. elegans,* the antioxidant effects of CAPE are related to an increase in lifespan and an improvement in the worms' quality of life quality (LI et al., 2021). Among the endpoints, CAPE could induce a stress response, the *hsp-3*, *hsp-16.1*, *hsp-16.41*, *hsp-17*, and *hsp-70* genes were negatively regulated. Simultaneously, the expression of *hsp-12.6, sir-2.1* and *sod-3* was significantly increased after exposure to polyphenol at specific concentrations, thus classifying CAPE as a hormetin (AYUDA-DURÁN et al., 2019; COLONNELLO et al., 2020).

Nevertheless, CAPE acts in a dependent way on DAF-16 and modulates its cell sub-localization, causing an accumulation of DAF-16 in the nuclear region(ZHANG et al., 2021). On the other hand, it did not change the sub-location of SKN-1 (HAVERMANN et al., 2014).

Feeding behavioral observations showed that CAPE increased the pharyngeal pumping and consequently the food ingestion. Thus, demonstrating that the antioxidant effects of CAPE are not due to caloric restriction, a result that corroborates the inability of this hormone to inhibit bacterial growth, which is a common property among polyphenols (PIETSCH et al., 2012).

The polyphenol increased the size of the litter in the first larval stages, a factor that can increase the chances of survival due to reorganization and redistribution of energy, to focus this energy expenditure on your maintenance functions. Other critical physiological changes noted after treatment with CAPE were a reduction in lipofuscin levels during the third and sixth days of adulthood and improved motor changes not dependent on age (PIETSCH et al., 2012).

Regarding the antioxidant activity, polyphenol showed significant activities both in hydrophilic and lipophilic solvents in N2 worms using the photoquimiluminescence (PCL) technique that consists of the optical excitation of a photosensitizer that results in the generation of the O_2^-, radicals that were reduced after treatment with CAPE. The *mev-1* mutant worms exposed to paraquat and then

treated with catechol showed an increase in survival compared to control groups, although the same feat was not observed in the *osr-1*, *sek-1* mutants, *sir-2.1*, *unc-43*, and *skn-1* (PIETSCH et al., 2012).

8. Conclusion and Future Directions

The aging process is inherent to humans and is usually accompanied by some molecular processes, such as the increase in ROS, which are cytotoxic and can negatively affect longevity.

However, in *C. elegans,* several plant products can reduce ROS levels and increase life expectancy in this model. Such studies have shown that these extracts can perform antioxidant functions through different mechanisms, with the main ones being the positive regulation or activation of antioxidant signaling pathways, such as IIS, which due to its highly conserved pathway, may indicate that similar effects of some plant-derived compounds would be observable in other organisms, including humans.

Moreover, some of these compounds play an antioxidant or pro-longevity function regardless of the participation of some of the signaling pathways, indicating a possible direct action on reactive oxygen species or in other mitochondrial damaging mechanisms.

In addition, studies have also demonstrated the role of these extracts in combating protein aggregates capable of inducing toxicity, mitophagy, and autophagy, mainly due to animal models of age-related neurodegenerative diseases. The plant products discussed herein are promising therapeutic targets for pathological processes involved in aging progress in humans.

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Conflict of Interest

Authors have declared no conflict of interest.

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Daiana Silva de Ávila
Priscila Gubert
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