



Review

Can *Caenorhabditis elegans* Serve as a Reliable Model for Drug and Nutraceutical Discovery?

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Abstract: Experimental research demands the selection of appropriate models to align with study objectives and conditions. Traditional experimental models, such as in vivo animal studies and in vitro systems like organoids, present nutraceutical and pharmaceutical research limitations such as high cost, ethical concerns, long lifespan, and difficult genetic manipulation. *Caenorhabditis elegans* has proved to be a valuable model as a result of its genetic and physiological similarities to higher organisms, fully sequenced genome, short life cycle, and transparency. These features enable high-throughput screening, molecular pathway analysis, and lifespan and healthspan assays. *C. elegans* has significantly advanced the discovery of bioactive molecules with therapeutic potential, shedding light on aging, neurodegeneration, metabolic disorders, and immune responses. Its utility in pharmacokinetics and validation of nutraceuticals underscores its role in longevity and metabolic health research. Additionally, its conserved stress response, apoptosis, and pathogen recognition pathways facilitate the study of pharmacological interventions for inflammation, oxidative stress, and infections. This study evaluates the applicability of *C. elegans* as a model for in vivo screening, analyses its role in drug efficacy testing, and discusses relevant advancements, associated difficulties, and what to expect of *C. elegans* in research.

Keywords: *C. elegans*; drug identification; drug target; nutraceutical; pharmaceuticals



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1. Introduction

The emergence of new viruses and the prevalence of chronic illnesses have resulted in an increasing demand for pharmaceutical and nutraceutical products [1]. Moreover, the limited availability of medicines and the possibility of developing resistance is a major concern for human health. As a result, research on developing novel pharmaceuticals and nutraceuticals has become crucial [2–4].

Nutraceuticals are bioactive substances, often obtained from food sources, that provide health benefits outside regular nutrition regimes. Pharmaceuticals are substances that have been chemically produced or derived from chemical or biological sources. They are intended to prevent, diagnose, treat, and cure diseases [3]. Pharmaceuticals have traditionally been used to treat serious conditions and prolong life, while nutritional supplements improve the quality of life and attenuate illness [3,4]. To fulfill the expanding requirement for better understanding of these substances, low-cost, relevant, high-throughput

screening (HTS) technologies are required [5]. The key concern of therapeutic candidates is whether they exert the expected physiological effect at the site of action; hence, the impact of compounds must be investigated in a biological model. One such biological model is the use of in vitro cellular systems that best represent certain diseases' physiological and biochemical processes [6]. In developing new drugs, such in vitro systems have been argued to provide more accurate predictions of drug efficacy before animal tests or clinical trials, with applications focusing on target validation, safety, and industrial potential [7]. Therefore, this approach is currently up-scaling the quality of potential drug candidate nominations and guiding drug development success potentials [8–10].

Nonetheless, from prior research, the challenges associated with in vitro investigations include the inability to simulate how a pharmaceutical drug would interact with all of the chemicals and cell types found within a complex organ and how to overcome the dynamism of the body's ecosystem which has various cellular processes in constant communication [11]. To overcome these challenges, technologies based on more advanced co-culture technologies involving living cells in vitro are already emerging. There is currently a movement from two-dimensional cell cultures to three-dimensional cell cultures, a new approach that is more successful at capturing the physiological environment due to many queries about its efficacy [12]. Specifically, 2D cultures have numerous disadvantages, including disrupting the interplay that exists in the cells and outside the cell environments, coupled with changes in cell shape, polarity, and division mechanisms. These drawbacks prompted the development of models that are largely related to in vivo situations. A typical example of such an approach is three-dimensional culture [13].

This innovative method can provide three-dimensional architecture and retain the cell heterogeneity found in vitro (Figure 1). However, there is a need to examine its peculiarities, potentials, and possible pitfalls holistically. It is important to examine closely its implications on the overall applications of in vitro systems. For instance, organoids are three-dimensional in vitro cell preparations that can better mimic disease heterogeneity, physiological function, gene specificity, and other features [14]. However, the upregulation of certain protein biomarkers in malignancies was not replicated in organoids. In addition, organoids could only be propagated for 3 weeks before the first passing, and the absence of a well-developed vascular system remains a crucial factor that has impeded the interpretation of drug tests [14,15]. Also, in studies related to congenital disorders, primary organoids may only be derived from pregnancies that have been terminated, which limits prenatal research into fetal development and congenital disorders [16]. It is crucial to remember that experimental models each have their unique set of strengths and limitations [17]. A specific study's unique research questions and objectives determine the chosen experimental model [17,18]. The organism *Caenorhabditis elegans* has shown a lot of desirable characteristics such as simplicity, the possibility of genetic manipulation, evolutionary conservation, amenability, and its use in high-throughput screening (HTS) [19]. This points to the need for an increase in the popularity and usage of *C. elegans* as a more trusted in vivo research model in relevant research (Figure 2).

Several reviews exist attempting to highlight the role of *C. elegans* in pharmaceutical development. O'Reilly et al. [19] trace the evolution of *C. elegans*-based drug screening, addressing the challenges associated with its use and highlighting recent technological advancements that have enhanced its potential for future drug discovery. Huang et al. [21] illustrate the advantages of *C. elegans* in high-throughput screening (HTS) for animal models. They further explore the mechanisms of candidate compounds, emphasizing their transformative role in translational research for drug discovery and development. Zarroug et al. [22] provide key insights into in vivo antimicrobial efficacy and the toxicity of natural products. It compares their evaluation in conventional vertebrate models. This under-

scores the value of *C. elegans* as a powerful model for identifying promising antimicrobial agents. Giunti et al. [23] emphasize the significant role of *C. elegans* in the discovery of diverse candidate compounds, particularly in research areas such as neurodegeneration, pathogenic infections, and metabolic disorders. Similarly, Kropp et al. [24] discuss how *C. elegans* contributes to the understanding of rare diseases and supports the development of potential treatments. Unlike these cited studies, our study focuses on using *C. elegans* in age-related decline, oxidative stress, and its critical role in longevity and healthspan research. We also provide mechanistic insights, including response pathways, beyond its use in basic drug screening, providing strong implications for its use in drug, nutraceutical, and dietary interventions.

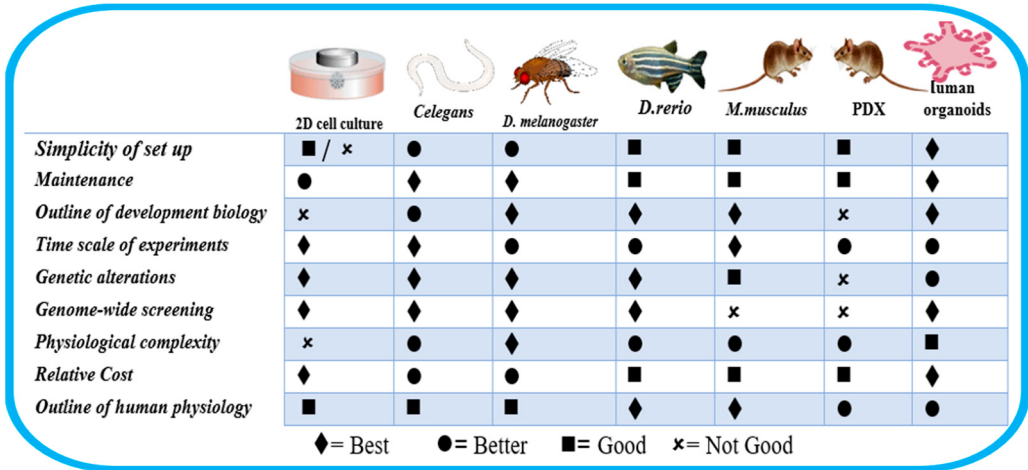


Figure 1. Characteristics of some cell lines and animal models. Adapted from Kim et al. [20].

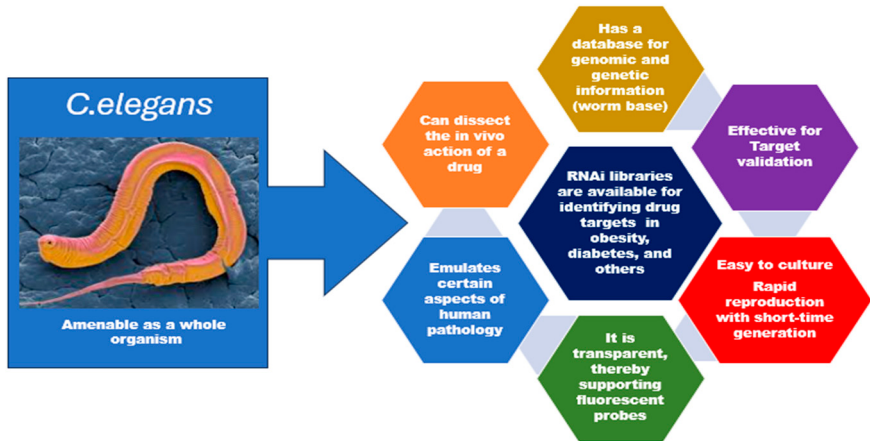


Figure 2. Desirable characteristics of *C. elegans* for pharmaceutical and nutraceutical research.

C. elegans can be used as an animal HTS model beyond the limitations of 2D cell systems or 3D organoids [25] (Figure 1) because proteome analysis comparing *C. elegans* and human protein sequences has revealed that human genetic homologs are present for 83% of the *C. elegans* proteome [26]. Moreover, *C. elegans* preserves biochemical and molecular biological pathways similar to those of humans. This review will offer a deeper comprehension of the use of *C. elegans* in evaluating medication effects and discovering their mechanisms in relevant pathways [14].

Section 1 introduces *C. elegans* and its desirable characteristics over other models, while Section 2 highlights its role in early drug development and target identification. Sections 3 and 4 explain its role in elucidating action mechanisms and pharmacokinetics. Sections 5 and 6 delve into the various roles of *C. elegans* in nutraceutical development and

antiaging research, highlighting gene identification, dietary restriction, specific gene mutations, cellular pathways, and the development of medications for age-related disorders. Sections 7–10 elucidate the role of *C. elegans* in screening antimicrobial compounds, lipid quantification, antiobesity research, neurodegenerative diseases, and gut health permeability research. Sections 10 and 11 look into its limitations and associated toxicity issues, followed by our conclusion.

1.1. Molecular Basis for Using *C. elegans* in Biomedical Research

The small roundworm *Caenorhabditis elegans* is a helpful model organism in drug investigation because of its physiological similarities to humans and other higher species, its genetic tractability, and its simplicity [27,28]. In this sense, several biochemical and genetic traits support its efficacy: it possesses numerous important biological pathways and molecular mechanisms that humans and *C. elegans* share that have evolved throughout time. In both animals, genes related to development, aging, neurology, and illness frequently have functional similarities [23]. As a result, knowledge about these processes in *C. elegans* may be applied to humans [29,30]. The study of cellular signaling pathways, organ development, and medication effects at the cellular level is made easier by its straightforward architecture, which consists of well-defined cells and tissues and its transparent body [19,31]. Hence, microscopy tools can easily visualize interior structures and cellular processes. Additionally, it possesses about 302 neurons and a thoroughly mapped network of connections of a comparatively simple nervous system. The genome of *C. elegans* is well-annotated, and strong genetic tools are available for its modification [26]. Precise control of gene expression and functions is made possible by methods like transgenesis, RNA interference (RNAi), and genome editing, making it possible for researchers to investigate how particular genes affect drug responses and disease characteristics. Moreover, *C. elegans* has a reproduction time of three days and yields a vast number of progenies, approximately 300 eggs for each hermaphrodite adult [32], thus facilitating fast and affordable large-scale screening assays to find possible drug candidates or investigate drug toxicity. Due to its simplicity, the worm is a great model for research in various fields such as neurobiology, particularly in terms of how medicines affect the behavior and function of neurons [19,27,28,32].

1.2. Is *C. elegans* Suitable as a Reliable Model?

Although imperfect, it has been considered an ideal model for various studies [33]. In comparison to humans, *C. elegans* has simpler biology. This implies that results might not always apply to humans directly. *C. elegans* also lacks some human organs. Therefore, it may not be the best model to investigate complicated disorders or those strongly influenced by those organs, and the nutritional research results may not immediately apply to *C. elegans* because it has different dietary requirements from humans. Also, *C. elegans* does not perfectly recapitulate the complete pathophysiology of vertebrates [34]. Although this experimental model has several drawbacks, *C. elegans*' ability to construct an overexpression model of any gene, in a single or combined state, and to conduct toxicology, recovery, or survival trials in short periods and at low cost is desirable. A continuous increase in its employment is expected [19,27,34].

The systematic combination of its genetic amenability, simplicity, and significance to human biology makes *C. elegans* a highly versatile and reputable standard model organism for several research areas, including developmental biology, neuroscience, genetics, and drug discovery [35]. It has found application in modelling various diseases such as high blood glucose, cardiovascular diseases, hyperlipidemia (obesity), and neurodegenerative diseases, it has helped to provide critical clues into the underlying systems of these diseases as well as unravel the beginning and progression of such illnesses [36]. The evolutionary

conservation of *C. elegans* and humans has paved the way for identifying novel therapeutic targets, whereby prospective therapeutic targets are validated in vivo.

2. *C. elegans* for Early Drug Development and Target Identification

Drug discovery can be viewed as a three-component equation in which bioactive chemicals are generated to act on molecular targets and change disease phenotypes [37]. As a result, drug development efforts begin with different experimental starting points, such as target identification or disease models explored by bioactive molecules. The principal target of most drugs is protein, since it is a major biochemical component of biological systems [38]. *C. elegans* has been used to expatiate the basic elements of pivotal biology processes, such as apoptosis, aging, and gene expression control, and to discover innovative drug targets and therapy in the pharmaceutical research sector [28].

Target validation is critical to any drug development effort [39]. It is a major challenge for post-genomic drug development, and selecting promising therapeutic targets from a large candidate pool seems outright impossible without a model system capable of reliably replicating in vivo processes and symptoms. To address this challenge, *C. elegans* has been leveraged as a well-characterized experimental tool for genetic research of biological regulatory networks [24,39]. Using this model system, it is possible to observe the impact of mutations on normal embryonic and post-embryonic development (before and after fertilization) and signal transduction pathways [28]. To this end, *C. elegans* is also used to model complex illnesses such as human diseases [40]. Leveraging its relatively small size and use of fluorescent SiNPs as low-toxicity probes, it has now become possible to observe the physiological and pathophysiological processes of living organisms [41–44]. This, in turn, simplifies the screening process of new pharmaceuticals and nutraceuticals for their impact on the intricate biological mechanisms involved in human illness; thus, *C. elegans* offers great potential for both target identification and early drug development [36].

Using a drug to modulate the activity of a specific target protein in an organism and track the effects over time has enabled the development of genetic screens for identifying the proteins and genes that may influence the pharmacological response [14]. In previous years, the process was largely manual and lacked scalability. However, recent developments in high-throughput liquid screening, image-based equipment, and data processing have made the cellular model a viable candidate for drug screening automation [45–47]. Unlike single-celled organisms and in vitro experimentation, *C. elegans* can simulate complex human diseases and further assess ADMET parameters (absorption, metabolism, distribution, excretion, and toxicity) at the initiation of drug discovery and then through the subsequent course. Additionally, the nematode contains various phenotypes that can be measured, and its multicellular structure offers further advantages [48]. Moreover, *C. elegans* has existing genetic tools and genomic resources [45,48].

C. elegans also possesses easily accessible, time-tested genetic tools and genomic resources (such as the RNAi-feeding library), allowing for greater precision in identifying drug targets and their putative mechanisms of action.

High-throughput drug screening assays are primarily centered around image-based phenotyping, with a diverse extent of throughput resolution and additional requirements for specialized tools such as microfluidic devices and worm trackers [49–52]. These assays mainly focus on morphological-descriptive characteristics of *C. elegans* while giving limited attention to vital locomotor parameters, which are crucial for screening drugs for muscle-related disorders [48]. Significant achievements in fields such as neurodegeneration, cancer, and metabolic diseases emphasize the relevance of this approach in applied research. Ongoing progress in genetic methodologies, combined with multi-omics technology, is set to further increase the utility of *C. elegans* in identifying new therapeutic targets [34].

3. *C. elegans* for Evaluation of Drug Effects and Possible Action Mechanisms

C. elegans self-fertilizes quickly, has a brief lifespan, and a straightforward maintenance routine in the laboratory [44]. This facilitates the easy elucidation of several metabolic pathways. Using *C. elegans* has enabled the examination of behavioral effects (activity, feeding, and chemotaxis) at different magnitudes of pharmaceutical and nutraceutical intake and the establishing of a dose-dependent relationship of various active ingredients and bioactive substances [48]. Furthermore, *C. elegans* is particularly relevant for studying aging and other metabolic processes due to its distinct life stages that can be physiologically and genetically examined [53–55]. The highly conserved insulin/IGF-1 signaling system (*daf-2/daf-16*) in *C. elegans* has been critical in aging research, suggesting therapeutic targets for healthy aging. For example, studies in *C. elegans* have demonstrated that inhibiting the mTOR pathway (a cellular regulator of growth and metabolism) increases lifespan. These discoveries have influenced drug development efforts, with molecules such as rapamycin being investigated for their anti-aging and therapeutic potential in age-related disorders [56–58].

In cancer research, *C. elegans* is a biosensor for general cancer screening [59]. It has conserved genetic pathways involved in cell cycle control and apoptosis, making it a useful model for discovering potential cancer targets. In the apoptosis pathway, the identification of the fundamental apoptotic machinery in *C. elegans*, which includes *ced-3*, *ced-4*, and *ced-9* (homologous to human caspases and *BCL-2* family members), has identified targets for drugs aimed at influencing cell death in cancer [60]. Unlike in *Drosophila* and mice, where apoptosis occurs via proteolytic cascades of initiator and executioner caspases, *CED-3* is the only major caspase required for apoptosis in *C. elegans* [61]. Specifically, *BH3* mimetics that inhibit anti-apoptotic proteins in cancer cells were developed using insights from *C. elegans* research [62]. Moreover, genes such as *HCP-1*, *HIM-10*, *ZW10*, *CENP-A*, and *CENP-C* involved in chromosomal segregation have been examined in *C. elegans* for a proper understanding of their involvement in genomic stability [63]. Dysregulation of these pathways has influenced the development of cancer treatments that target mitotic checkpoints.

C. elegans has been used to study the pharmacodynamics and drug–drug interactions of anthelmintic ivermectin (IVM) and eprinomectin (EPM) [55]. It has also been used as a promising model in validating novel effective medications in treating helminth infections and elucidating the mechanism of action of anthelmintic compounds [5]. In addition, high-throughput behavioral screens of *C. elegans* have exposed potential drug candidates for Parkinson’s disease (PD), emphasizing the utilization of high-throughput platforms and methodology that evaluate the automation of *C. elegans* postures [42]. The nematode has also assisted in investigating dose, exposure time, and drug–drug interactions [64–66]. A *C. elegans* model for Amyotrophic Lateral Sclerosis (ALS) has been developed using the *dnc-1* KD approach. Incorporating the Multi-Worm Tracker (MWT) with the *dnc-1* KD model creates a behavior-based, automated, and quantitative method for drug screening [56,67,68]. *C. elegans* has also provided insights into the action mechanism of Metformin by modifying microbial folate and methionine metabolism through the lysosomal pathway [69,70]. Additionally, it has contributed to understanding dopaminergic degeneration in neuroprotection and the progression of Alzheimer’s disease [71–73]. Synaptic transmissions and damage, the cause of cognitive impairment in Alzheimer’s disease, have been widely studied using *C. elegans*. A resveratrol-loaded nanostructured lipid carrier that increased acetylcholine concentration at the synapses was employed to treat Alzheimer’s disease using *C. elegans* [73]. Interestingly, aldicarb, a carbamate of acetylcholine esterase inhibitors, is known to induce paralysis in *C. elegans* by persistently activating ionotropic acetylcholine receptors. The severity of paralysis and the rate at which it develops are dependent on

the dosage. RNA-mediated interference has revealed that mobility assays apply to probes related to both genetic and drug screening. Essentially, *C. elegans* presents a viable choice for drug screening and the research assessment of pharmaceuticals and other genetic and RNAi-mediated research [74].

4. *C. elegans* in Pharmacokinetic Studies

Pharmacokinetics examines the absorption, distribution, associated metabolism, and excretion of drugs and related substances (ADME). Traditionally, it relies on mammalian and in vitro models [48]. However, *C. elegans* has become visible as an affordable, genetically tractable alternative [75]. Despite its simplicity, it shares key drug metabolism pathways with mammals, including CYP enzymes, ABC transporters, and detoxification mechanisms, making it valuable for high-throughput pharmacokinetic research [75–77]. Interestingly, utilizing *C. elegans* in pharmacokinetic research goes beyond typical studies, as advances in microfluidic technologies now allow for real-time monitoring of medication uptake and metabolism within individual worms. Additionally, genetically engineered strains expressing human CYP enzymes can potentially upscale the prediction accuracy of drug metabolism investigations [77]. Table 1 highlights the pharmacokinetic applications of *C. elegans* in drug discovery.

Researchers identified several metabolic pathways in *C. elegans*, including the formation of glucoside metabolites for drugs like albendazole and genistein, which appear to differ from mammalian metabolism. Amirthagunabalasingam et al. [51] developed an epifluorescent microscopy approach to monitor doxorubicin uptake into the pharynx of *C. elegans*, highlighting the role of organic cation transporters in this process. The accumulation of fluorescein, an organic anion, in the intestinal lumen and cells of *C. elegans* has demonstrated the functionality of organic anion transporters (OATs) in this organism. However, Hartman et al. [75] noted the presence of physical barriers to chemical uptake in *C. elegans*, emphasizing the importance of considering these factors in pharmacokinetic studies. Fluorescent tracers and high-performance liquid chromatography (HPLC-MS/MS) have enabled real-time tracking of drug absorption and distribution within the worm's tissues [78]. Hence, various compounds, including anticancer drugs, anthelmintics, and antidepressants, have also been tested using methods such as HPLC-MS/MS and fluorescence microscopy to track drug uptake and metabolism. However, important limitations were noted, including how environmental factors (such as *E. coli* presence) affect drug processing and how *C. elegans*' metabolism differs from that of mammals. Accordingly, Van der Most et al. [79] highlighted the influence of environmental factors, such as the presence of *E. coli* as a food source, on the uptake and elimination of fluoxetine in *C. elegans*. While *C. elegans* offers advantages like easy genetic manipulation and quick experiments, the distinct metabolic pathways observed suggest results may not directly translate to higher organisms, indicating that *C. elegans* can be valuable for initial drug metabolism research, but additional testing in more complex organisms remains necessary for drug development applications [19,75].

A major advantage of *C. elegans* is its ability to model drug absorption. The worm's intestine, its primary site of drug uptake, allows researchers to study how compounds permeate biological membranes. Studies have shown that lipophilic drugs, such as rifampicin, are more readily absorbed, whereas hydrophilic drugs, such as metformin, exhibit limited uptake trends that mirror those observed in mammalian models [77]. Beyond absorption, *C. elegans* is also a valuable tool for studying drug metabolism. Its xenobiotic detoxification pathways include phase I modifications via CYP enzymes and phase II conjugation through UDP-glucuronosyltransferases (UGTs) and glutathione-S-transferases (GSTs) [76,77]. For instance, Shen [80] demonstrated that acetaminophen and nonsteroidal anti-inflammatory

drugs (NSAIDs) undergo a metabolic transformation in *C. elegans* through homologs of human CYP1A2 and CYP3A4. These findings propose that *C. elegans* can be used to predict drug metabolism patterns before progressing to mammalian studies. The excretion of drugs in *C. elegans* further highlights its pharmacokinetic relevance. Efflux transporters, particularly P-glycoprotein (PGP-1), are crucial in drug clearance. Studies have shown that *C. elegans* mutants lacking PGP-1 exhibited increased doxorubicin accumulation, thereby reinforcing the importance of ABC transporters in drug efflux. This ability to study transporter-mediated drug excretion provides valuable insights into mechanisms of drug resistance and clearance [76,77].

While it does not fully replace mammalian models, it is an efficient preclinical tool for early-stage pharmacokinetic screening, helping to optimize drug candidates before transitioning to more complex systems. The progressive development of more advanced technologies, such as integrating artificial intelligence and machine learning models with *C. elegans* pharmacokinetic data, could further refine drug screening pipelines, reducing reliance on costly mammalian trials.

Table 1. Examples of pharmacokinetic application of *C. elegans* in drug discovery.

S/N	Technical Approaches	Applications	Drug Class	Limitations	Ref
1	Fluorescence microscopy and plate reader	Quantifying organic anion uptake and accumulation	Organic anions (Fluorescein)	Limited to fluorescent compounds	[51]
2	Epifluorescent microscopy	Quantifying organic anion uptake and accumulation	Anticancer (Doxorubicin)	Limited to fluorescent compounds	[52]
3	In-silico docking and ADMET analysis	Predicting drug interactions and properties	Herbicide (Paraquat), Antiparkinson (Levodopa)	Requires experimental validation	[81,82]
4	High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS)	Metabolite identification and characterization	Anthelmintic (Albendazole)	Requires specialized equipment	[83]
5	Whole genome microarrays, Reverse Transcription Quantitative Polymerase Chain Reaction (RT-QPCR), HPLC-MS	Gene expression analysis and metabolite identification	Anthelmintics (Ivermectin, Albendazole)	Complex data interpretation	[84]
6	Liquid Chromatography with Diode Array Detection (LC-DAD) and LC-MS/MS	Detailed metabolite characterization	Isoflavone (Genistein)	Requires specialized equipment	[85]
7	Toxicokinetics experiments, one-compartment model	Studying drug absorption, distribution, metabolism, and excretion	Antidepressant (Fluoxetine)	Model assumptions may not fully represent biological complexity	[79,82]
8	Behavioral analysis	Assessing drug effects on nematode locomotion, and potential resistance.	Anthelmintic (Pyrantel)	Indirect measure of pharmacokinetics	[76,85]

5. *C. elegans* and Nutraceutical Development

Nutraceuticals are bioactive food products that present additional health advantages beyond regular nutrition [86]. These compounds are sometimes linked to oxidative stress reduction, preventing inflammation, and promoting longevity [86,87]. This model organism functions as a valuable tool for researchers exploring the mechanistic effects of nutraceuticals [88].

A key strategy in nutraceutical development using *C. elegans* involves examining the impact of bioactive components on the worm's healthspan, lifespan, and overall physiology [89]. *C. elegans* plays a crucial role in uncovering genetic pathways linked to various physiological processes and assessing the influence of nutraceuticals on these pathways. Notably, two well-characterized pathways regulating these processes in *C. elegans* are the transforming growth factor- β (TGF- β) and insulin/insulin-like growth factor-1 (IGF-1) pathways. These pathways provide critical molecular insights into the mechanisms underlying numerous pathological conditions and support the development of novel nutraceuticals [89].

Strong evidence supports the potential of nutraceuticals, such as chondroitin sulfate, in developing therapeutic agents for Alzheimer's disease. Studies using transgenic strains of *C. elegans* have shown their ability to protect against oxidative stress and A β -induced toxicity [90,91]. The neuroprotective effects of certain nutraceuticals have also been well-documented. For instance, epigallocatechin-3-gallate, found in green tea and rapeseed pomace, has been shown to suppress neurodegeneration, while *Grifola frondosa* contributes to reduced fat accumulation and extended lifespan through the DAF-16/FOXO and SKN-1/NRF signaling pathways. These effects have been extensively studied in *C. elegans*, particularly with insulin signaling and other regulatory pathways [92]. Additionally, *C. elegans* serves as a valuable model for screening potential nutraceuticals by assessing their effects on specific phenotypes. Research has identified bioactive compounds with antioxidant properties that enhance mitochondrial function and promote healthy aging. These findings pave the way for developing novel anti-aging compounds [93,94].

Bioactive compounds are often assessed for bioactivity by evaluating a variety of phenotypes, including a significant reduction in growth rate, lethality, uncoordinated movement, and other morphological defects in wild-type or mutant animals. *C. elegans* worms show a variety of intrinsic behaviors, including chemotaxis toward attractive signals, aversion to unpleasant stimuli, and mating. Immune cells employ chemotaxis to move between the vascular and lymphatic systems and migrate from the blood to infection sites [95,96]. This appears to be a reliable strategy for studying the behavioral patterns of bioactivity in nutraceuticals. A major bottleneck worthy of note is in the administration of hydrophobic compounds to *C. elegans*, as highly hydrophobic nutraceuticals such as curcumin are prone to precipitation when added to aqueous media containing *C. elegans* growth medium, often resulting in low and varied levels of bioaccumulation in *C. elegans*, thereby complicating the standardization of experiments and the interpretation of the results during nutraceutical development and analysis [96].

6. *C. elegans* in Pharmaceutical Development in Anti-Aging Research

6.1. *C. elegans* in the Identification of Genes That Affect Lifespan

C. elegans is used in aging, age-related disorders, longevity, and drug screening, owing to its well-defined physiological characteristics with regard to aging [97]. Both genetic and environmental factors influence lifespan. Researchers have used *C. elegans* to identify genes that regulate aging, demonstrating that certain gene mutations can extend lifespan by up to 50%. Many of these genes are linked to key signaling pathways that connect aging to environmental conditions [98]. *C. elegans* has fundamentally shifted the understanding

of aging from a passive deterioration to an actively regulated process. One of the most significant findings was identifying the IGF-1 signaling pathway as a key controller of lifespan in *C. elegans*. This discovery provided critical insights into aging mechanisms and established insulin signaling as the first known pathway involved in lifespan regulation [99]. Research using *C. elegans* has also uncovered various anti-aging strategies, including dietary restriction, specific gene mutations, cellular pathways, and natural compounds that can slow aging.

Epigenetic modifications also play a pivotal role in the response to environmental stress. *C. elegans* can adapt to various stressors, such as heat and oxidative stress, through epigenetic changes that help protect against age-related decline. Researchers have utilized *C. elegans* to investigate how these epigenetic mechanisms are affected by factors such as diet, lifestyle, and pharmaceutical compounds. Notably, compounds that target epigenetic regulators, such as histone deacetylases (HDACs), are being explored for their potential to extend lifespan and promote healthier aging [99,100].

6.2. Dietary Restriction and Specific Gene Mutation

Aging significantly increases the risk of developing chronic diseases. One effective strategy for delaying age-related illnesses is dietary restriction (DR), which involves reducing food intake without causing malnutrition. Research across various organisms has shown that DR can extend lifespan, and model organisms like *C. elegans* are widely used to investigate the underlying mechanisms with potential therapeutic applications [101,102]. Studies in *C. elegans* have demonstrated that reducing calorie intake can substantially increase longevity, sparking further research into dietary interventions for lifespan and healthspan extension. However, in laboratory settings, *C. elegans* is typically fed attenuated *E. coli* bacteria (OP50) on agarose plates [102]. To this end, Greer et al. [101] identified several distinct DR regimes in *C. elegans*: A genetic mutation (*eat-2*) that reduces pharyngeal pumping, thereby decreasing food intake; bacterial dilution in liquid cultures (bacterial DR (bDR) and liquid DR (IDR)); two chemically defined liquid media—axenic medium and chemically defined liquid medium (CDLM)—which mimic DR-like effects; peptone dilution on agarose plates (DP), which inhibits bacterial growth; complete absence of bacteria on plates, referred to as dietary deprivation (DD); and a recently developed approach which requires the serial dilution of bacteria on plates, known as solid DR (sDR).

Lowering metabolic rate and reducing IGF-1 signaling are two proposed mechanisms through which dietary restriction (DR) extends lifespan [103]. While experimental data on this topic remain limited, interaction studies suggest that DR and insulin signaling may function in parallel within similar regulatory systems [104,105]. Additionally, evidence indicates that the target of rapamycin (TOR) pathway is responsible for the impact of DR on longevity in *C. elegans* [106]. This suggests that DR's impact on lifespan likely involves multiple metabolic pathways, which may vary depending on the specific DR regimen used and the organism studied [101].

Certain gene mutations in *C. elegans* have been found to prolong lifespan, in some instances nearly doubling it. These mutations often enhance stress resistance, improve protein quality control, or optimize metabolic efficiency [101].

6.3. Natural Chemicals and Cellular Pathways

Natural compounds that target conserved cellular pathways pave the way for the development of novel nutraceuticals and pharmaceuticals to promote longevity and healthspan [107]. Researchers have identified numerous anti-aging substances by feeding *C. elegans* various natural compounds, including antioxidants, sirtuin activators, and rapamycin. For instance, *Ganoderma lucidum* promotes oxidative stress resistance and extends

lifespan in *C. elegans*. Its therapeutic effects may be mediated through multiple signaling pathways [108].

Additionally, administering *Momordica* saponin extract to both stressed and normal *C. elegans* increases the expression of key antioxidant enzymes [109]. Studies across various model organisms also suggest that curcumin supplementation extends lifespan by enhancing SOD activity while reducing levels of malondialdehyde (MDA) and lipofuscin, both of which are markers of oxidative damage and aging [110–113].

Curcumin also alters critical signaling pathways that regulate organism lifespan, such as the IIS, mTOR, PKA, and FOXO signaling pathways. It also induces HSP expression, enhances oxidative stress resistance, reduces ROS accumulation, and promotes autophagy [110], thus enhancing motility, healthspan and lifespan in *C. elegans*. Berberine, epigallocatechin gallate, and quercetin are other natural substances for which mechanistic studies have revealed their lifespan-promoting effect in *C. elegans* through the modulation of the IIS pathway, AMPK activation, improvement of mitochondrial function, inhibition of the TOR pathway, induction of autophagy, and activation of DAF-16. Undeniably, *C. elegans* research has shed light on several cellular pathways that play critical roles in aging, including the insulin/IGF-1 signaling system, the mTOR pathway, and the unfolded protein response [109,114–116].

Various methods of modifying nutrition and chemical substances increase longevity via separate and non-linear genetic pathways. Numerous peptides interacting with the insulin/IGF-1 receptor regulate the IIS pathway in invertebrates. The IIS pathway in *C. elegans* includes several proteins encoded by some genes, including *daf-18* (encoding the human tumor suppressor PTEN homolog), *daf-2* (mammalian insulin/IGF-1 receptor homolog), *age-1* (PI3K homolog), and *akt-1* (serine–threonine protein kinases) [109]. The reduced activity of the genes *age-1*, *akt-1*, and *daf-2* was demonstrated to down-regulate this signaling cascade, and worms with mutations in these genes were proven to live longer. This important finding that lower insulin/IGF-1 signaling (IIS) levels can elicit lifetime extension in the worm *C. elegans* was published 24 years ago [109]. Alterations in the insulin/IGF-1 receptor gene *daf-2* or other genes in this pathway can double longevity in this organism. On the other hand, activating the IIS pathway shortens the lifespan of *C. elegans*. Resveratrol notably increased the longevity of wild-type worms by a small but statistically significant amount. Resveratrol, on the other hand, did not extend the lifetime of *aak-2(ok524)* mutant worms, suggesting that AMPK is required for resveratrol's favorable effects on longevity [103,114]. AMPK activity is not necessarily linked to FoxO activation, even though AMPK's capacity to increase longevity is dependent on the presence of FoxO. Resveratrol has been shown to extend the lifespan of *daf-16(mu86)* mutant worms, meaning that its longevity-promoting effects occur through an AMPK-dependent but FoxO-independent pathway [103,114]. The improved stress resilience and health-promoting effects associated with *Momordica* saponin extract (MSE) in *C. elegans* may stem from its dual function as a direct free radical scavenger and a regulator of signaling pathways involved in stress adaptation. MSE-mediated stress resistance appears to include the insulin signaling (IIS) pathway [109,115].

6.4. Developing New Drugs to Improve Age-Linked Diseases

A critical part of aging research is the quest for drugs that promote lifespan and healthspan, followed by a thorough examination of their mechanisms of action. However, identifying both medications and their targets is difficult. *C. elegans* is an appropriate model for such studies due to its short lifetime and genetic tractability [115]. By suppressing 18S rRNA/ribosome, fatty acid amide hydrolase-4, Hsp90, and the Ca²⁺-activated K⁺ (BK) channel SLO-1, minocycline, JZL184, monorden, and paxillin have been shown to

improve the lifespan of *C. elegans*. [116,117]. In several animal species, including *C. elegans*, decreasing mRNA translation and protein synthesis increases lifespan. Rapamycin, which reduces mRNA translation by blocking the mechanistic target of rapamycin (mTOR), increases lifespan and healthspan in various organisms from yeast to mammals. As a result, new chemicals that inhibit protein synthesis are attractive antiaging therapeutic prospects. In recent times, numerous antiaging compounds have been identified through the use of *C. elegans* models [117,118]. It has been used to study drug development that can reduce lipofuscin accumulation, a pigment thought to contribute to aging [118,119]. Because many of the processes that govern aging and longevity in *C. elegans* are evolutionarily conserved, these newly discovered lifespan-extending chemicals may help lead the development of antiaging drugs for human applications.

C. elegans has been utilized as an organismal model to assay for survival against a range of mitochondrial inhibitors by measuring worm longevity and aging indicators such as pharyngeal muscle contraction, pigment buildup, lipofuscin, and ATP levels [120]. *C. elegans* has thus helped in the development of FDA-approved medications and nutritional alternatives that not only elongate but also improve the overall quality of life [120–122].

Organisms constantly experience environmental fluctuations and disruptions in intracellular homeostasis, which can negatively impact their proteome and overall physiology [123]. To subdue these constraints, they have evolved specialized and highly regulated stress responses that repair cellular damage and maintain homeostasis. These protective mechanisms include the heat shock response (HSR), the unfolded protein response in the endoplasmic reticulum, the unfolded protein response in mitochondria, and the oxidative stress response [123]. Research on stress resistance provides valuable insights into how internal and external stressors influence biological processes such as cellular homeostasis. The ability of *C. elegans* to withstand various abiotic stressors, including oxidative stressors, hypoxia, hyperoxia, extreme temperatures, osmotic stressors, ultraviolet (UV) radiation, endoplasmic reticulum (ER) unfolded protein stress, and heavy metal toxicity, has significantly contributed to our understanding of complex disease pathways [124].

Scientists have utilized *C. elegans* to investigate how various stresses impact aging. For example, studies have unveiled that exposure to oxidative stress may reduce lifespan. Research into stress responses in *C. elegans* has uncovered a range of complex processes and disorders [124]. However, there is a lack of consensus in the heat shock/heat stress field regarding the optimal timing, temperature, and duration of exposure, leading to significant variation in experimental protocols across different laboratories. This lack of standardization makes results comparison a Herculean task across studies. Determining the best approach when developing a new protocol has also become increasingly difficult [125]. The desire to understand how cells respond to physical and chemical stressors has increased interest in stress proteins as potential toxicological endpoints. In this context, transgenic strains of *C. elegans* have been created, featuring a reporter enzyme controlled by a stress-inducible promoter [126]. This reporter is easily measurable in live nematodes and responds to several chemical stimuli, making transgenic *C. elegans* a valuable tool for developing rapid, accessible, and informative bioassays.

7. *C. elegans* in Screening Novel Antimicrobial Compounds

The rise of antibiotic resistance and the urgent need for novel antimicrobial agents have driven researchers to explore alternative high-throughput screening (HTS) models for drug discovery [127]. *C. elegans* infection models allow researchers to study host–pathogen interactions, drug efficacy, and toxicity within a living organism. Although *C. elegans* lacks an adaptive immune system, its innate immune responses are similar to mammalian immunity, including TLR signaling, MAPK pathways (e.g., p38 MAPK in

pathogen response), and antimicrobial peptide (AMP) synthesis [128,129]. Because of its conserved pathways, it is an ideal surrogate host for testing immune regulation by antimicrobial drugs. *C. elegans* may be cultivated on 24, 48, 96-well plates, and more, allowing for automated HTS of chemical libraries [130]. Also, *C. elegans* investigations require 100–1000 times lower medication doses than mammalian models, resulting in significant cost reductions [5].

C. elegans models can help identify new antimicrobial compounds, including those that might work against antibiotic-resistant strains and persister cells [131,132]. These models appeared particularly useful in identifying compounds that work in living organisms but might be missed by traditional laboratory testing methods, possibly because they capture host and pathogen relationships. *C. elegans* models proved effective in identifying novel antimicrobial compounds across various studies. For instance, Escobar et al. [46] discovered N-[3,5-Bis(trifluoromethyl)phenyl]-5-chloro-2-hydroxybenzamide (IMD0354), a kinase inhibitor with potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

A total of 28 compounds not previously reported to have antimicrobial properties have been identified [133]. Samsudin [131] found synthetic retinoids CD437 and CD1530 effective against MRSA and persister cells. Other microbial strains used are Vancomycin-Intermediate *Staphylococcus aureus* (VISA), Vancomycin-Resistant *Staphylococcus aureus* (VRSA), and Methicillin-Susceptible *Staphylococcus aureus* [134,135]. Four novel anti-staphylococcal compounds with minimum inhibitory concentrations (MICs) ranging from 2 to 8 ff_g/mL against MRSA have been discovered [136]. These discoveries highlight *C. elegans* models' potential to identify compounds with novel mechanisms of action, the ability to modulate host defense, the capacity to target bacterial virulence factors, and effects beyond direct inhibition of bacterial growth.

The compounds identified through *C. elegans* screening show promising therapeutic potential (Table 2). IMD0354 demonstrated potent bacteriostatic activity against various vancomycin-resistant strains with low toxicity. Synthetic retinoids CD437 and CD1530 showed efficacy against both growing and persister MRSA cells, thus addressing a significant clinical challenge. Additionally, Brevinin-2 family peptides improved the survival of MRSA-infected worms and showed potential as new applied peptide candidates [137]. These studies report that *C. elegans* models identified compounds effective against antibiotic-resistant strains, which may be relevant to current clinical needs.

Table 2. *C. elegans* models in identifying novel antimicrobial compounds across various studies.

Study Design	Bacterial Strain Used	Screening Method	Key Findings	Compound Class	In Vitro Activity	In Vivo Activity	Clinical Potential	Ref
HTS	MRSA, VISA, VRSA, VRE	<i>C. elegans</i> -MRSA infection model in 384-well plates	Identified IMD0354 as a potent bacteriostatic drug against MRSA and VRE	Kinase inhibitor (IMD0354)	Minimum Inhibitory Concentration (MIC): 0.06–0.25 µg/mL against MRSA and VRE	Complete protection of <i>C. elegans</i> at 7.14 µg/mL	Promising lead compound for multidrug-resistant bacteria	[46]
<i>C. elegans</i> infection model	<i>B. subtilis</i> GS67	N/A	<i>B. subtilis</i> GS67 increased <i>C. elegans</i> resistance to Gram-positive pathogens, through the production of fengycin	Lipopeptide (Fengycin)	-	Increased worm resistance to pathogens	Potential for commensal mediated protection	[136]
Liquid-based screening assay	<i>S. aureus</i> NCTC8325-4, MSSA, ATCC33591 (MRSA)	Liquid-based <i>C. elegans</i> - <i>S. aureus</i> assay in 24-well plates	Identification of 14 natural compounds and 14 synthetic compounds that prolonged survival of infected worms	Natural extracts and synthetic compounds	>50% survival of infected worms	8/28 hits had no effect on <i>S. aureus</i> growth	Potential for identifying host-defense modulators	[138]
Automated HTS	<i>E. faecalis</i> MMH594	Automated <i>C. elegans</i> - <i>E. faecalis</i> model in 384-well plates	Identified 28 compounds not without prior antimicrobial properties	Various compounds and extract	Promoted nematode survival	Some compounds had little or no effect on in vitro bacterial growth	Potential for identifying compounds targeting bacterial virulence or host defense	[139]
HTS	Automated HTS	<i>E. faecalis</i> MMH594	Identified 28 compounds without prior antimicrobial properties	Various compounds and extracts	Enhanced survival of infected worms	Six structural classes had a positive effect on infected <i>C. elegans</i> but had no effect on pathogen growth in vitro	Novel antimicrobial compounds with potential host-mediated effects	[134]

Table 2. Cont.

Study Design	Bacterial Strain Used	Screening Method	Key Findings	Compound Class	In Vitro Activity	In Vivo Activity	Clinical Potential	Ref
High-throughput liquid screening	MRSA MW2 BAA-1707, VRSA VRS1	<i>C. elegans</i> -MRSA liquid assay in 384-well plates	Identified 27 out of 30 clinically used antibiotics and the antihelminthic drug closantel as hits	FDA-approved drugs	Increased <i>C. elegans</i> survival	Identified known antibiotics and novel antimicrobial compound (closantel)	Potential for repurposing existing drugs for antimicrobial use	[135]
<i>C. elegans</i> infection screen	MRSA	<i>C. elegans</i> -MRSA infection screen (method not specified)	Identified two synthetic retinoids, CD437 and CD1530, effective against MRSA and persister cells	Synthetic retinoids	Enhanced survival of infected worms	Effective against both growing and persister MRSA cells	Promising new class of antimicrobials for difficult-to-treat infections	[140]
HTS	MRSA MW2, <i>E. faecium</i> ATCC E007, <i>K. pneumoniae</i> ATCC 77326, <i>A. baumannii</i> ATCC 17978, <i>P. aeruginosa</i> PA14, <i>E. aerogenes</i> EAE 2625	<i>C. elegans</i> -MRSA HTS in 384-well plates	Identified four novel antistaphylococcal compounds with MICs from 2 to 8 ffg/mL against MRSA	Novel synthetic compounds	Improved survival of MRSA-infected worms	MICs: 2–8 ffg/mL against MRSA and <i>E. faecium</i>	New antistaphylococcal compounds with potential for further development	[136]
<i>C. elegans</i> infection model	MSSA (type-C and type-G strains)	<i>C. elegans</i> -MSSA infection model	Demonstrated correlation between MSSA strain virulence in <i>C. elegans</i> and clinical manifestations	Virulence assessment	N/A	Correlated with clinical manifestations	Potential for using <i>C. elegans</i> to predict clinical virulence of <i>S. aureus</i> strains	[141]
<i>C. elegans</i> infection assay	MRSA	<i>C. elegans</i> -MRSA assay	Identified four Brevinin-2 family peptides that improved survival of MRSA-infected worms	Brevinin-2 peptides	Improved survival of MRSA-infected worms	N/A	Potential new applied peptide candidates against MRSA	[135]

8. *C. elegans* in Lipid Quantification and Antiobesity

8.1. Genetic Regulators of Lipid Metabolism

The importance of using model systems that compare favorably with in vivo high-throughput genetic screening to relate genome-wide sequencing (GWAS) gene candidates to several clinical disorders is on the rise [142]. Obesity and its associated metabolic syndrome are a fast-rising public health challenge. Given its high impact on people and the peculiarity of the healthcare system, there has been a surge in interest in discovering pharmaceutical targets for obesity therapy and prevention [143]. Although *C. elegans* is evolutionarily distant from humans, the two species share key lipid, sugar, and protein metabolic pathways [144]. In both organisms, regulators like TOR kinase and AMPK, as well as transcription factors like Sterol response element binding Protein (SREBP), Peroxisome proliferator-activated receptor gamma (PPAR), and Transcription Factor EB (TFEB), regulate genes related to metabolism and cell responses when nutrients are available. In *C. elegans* and human systems, the loss of function of such regulators generates comparable metabolic dysregulation, such as obesity or resistance to it [142–144]. Although GWAS has been employed to identify variants in human genetics that are associated with obesity, there is a need for experimental assertion, which consequently provides a springboard and direction for its prevention and treatment. As a result, hundreds of human genes related to obesity have been identified and classified using *C. elegans* [145].

A similar study identified 14 genes that support obesity and three genes that inhibit diet-induced obesity when silenced. The holistic effect of this prevention results in the prevention of excess fat accumulation and an overall lifespan expansion in *C. elegans* [146]. The use of model systems for in vivo high-throughput genetic screening is becoming increasingly important for linking GWAS gene candidates to various clinical disorders [142].

8.2. Experimental Approaches and Applications of *C. elegans* in Lipid Antiobesity Studies

The model organism *C. elegans* has been extensively used to investigate lipid metabolism through a variety of techniques, such as imaging lipid droplets, analyzing total fatty acids, and utilizing lipidomics approaches to study complex lipids [146]. Several transcription factors have been identified as key regulators of lipid metabolism in *C. elegans*. For instance, the loss of *mdt-15*, a homolog of peroxisome proliferator-activated receptor-gamma coactivator 1 α , results in reduced fat storage in nematodes [147–149]. Additionally, *cebp-2* is important in regulating fatty acid mitochondrial β -oxidation and desaturation [148,149]. Increased expression of *nhr-49* and *ech-1.1* enhances mitochondrial β -oxidation of fatty acids. Furthermore, delta-9 desaturases, including *fat-5*, *fat-6*, and *fat-7*, regulate overall body fat by modulating fatty acid production [148–151].

In a study investigating the potential antiobesity effects of a natural product on *C. elegans*, Shen et al. [147] analyzed 18 lipid and glucose metabolism genes using an RT-qPCR assay. Their findings revealed that the citrus extract under consideration significantly reduced the expression levels of genes such as *mdt-15*, *tub-1*, *cebp-2*, *nhr-80*, *fat-7*, and *sbp-1*, to mention a few. These results suggest that the extract may inhibit lipid accumulation (*fat-5*, *fat-6*, *fat-7*, *fat-4*, *fat-2*, *sbp-1*), modulate transcriptional regulators involved in lipid metabolism, such as those linked to β -oxidation and fatty acid desaturation (*mdt-15* and *cebp-2*), and influence lipid signaling (*nhr-80* and *tub-1*). However, expression of *atgl-1*, *acs-2*, *aak-2*, *hosl-1*, *elo-2*, *ech-1.1*, and *nhr-49* did not show significant changes. Furthermore, *C. elegans* can synthesize various lipids, and lipid metabolic genes present in the worm have been identified by comparative genomics. In particular, 71 of these conserved genes are linked to human metabolic disorders. The various lipid classes identified in *C. elegans* have been reviewed by Witting et al. [146], as well as their biosynthetic processes and chosen biological and therapeutic functions.

Fatty acids are utilized as building blocks for complex lipids. They also function as signaling molecules, and fatty amides are crucial molecules for metabolic signaling, such as relating diet to lifespan extension [148]. In *C. elegans*, PUFAs are essential for proper neurotransmission, and the *C. elegans* mutant *fat-3* causes functional but not developmental nervous system abnormalities and inadequate neurotransmitter release [148,149]. Comprehensively, the central nervous system plays a crucial role in regulating energy balance. Studies aimed at understanding the physiological mechanisms of body weight management identified dopamine as a key regulator of energy balance [150–152]. Knock-out mice with impaired dopamine function consume significantly less food and succumb to malnutrition within a few weeks after birth. Additionally, hormones such as leptin, insulin, and ghrelin, which relay the energy state of the peripheral nervous system to the CNS, act on dopaminergic neurons [153–155]. Genetic studies of fat storage in *C. elegans* exposed to varying dopamine concentrations have identified potential molecular targets involved in fat deposition and provided insights into the effects of dopamine signaling on fat accumulation [154]. Notably, the exposure of *C. elegans* to exogenous dopamine resulted in a dose-dependent decrease in fluorescent signals, a response that was reversible upon termination of the dopamine treatment [149].

Like in other animals, fat regulation in *C. elegans* reflects the consequence of behavioral, physiological, and metabolic processes. Its amenability to testing has led to its use in unraveling the complicated homeostatic systems that underpin energy balance in whole animals. This, coupled with its optical advantages, has allowed for the investigation of cell biological mechanisms of fat absorption, transport, storage, and usage [149,155]. Homogenates from *C. elegans* have been used to access phospholipid and triglyceride levels by diverse chromatographic methods. Various methodologies for *de novo* fat synthesis, fatty acid uptake, and fatty acid breakdown can now be adequately investigated using different *C. elegans* strains, thereby expanding the scope of fatty acid and obesity research [149].

8.3. Applications in Obesity Research

Obesity has become a global epidemic, affecting approximately one-third of the United States population. It is currently a public health challenge in terms of its prevalence and the escalating economic burden it imposes on healthcare systems [154]. The *C. elegans* model is a reliable tool in advancing our understanding of obesity and its underlying molecular mechanisms. *C. elegans* has been extensively employed to explore critical pathways in obesity research, particularly in insulin signaling, dietary glucose response, and the role of serotonin in regulating satiety, eating behaviors, and hypoxia-related metabolic disorders [104]. Its use in studying how dietary factors influence metabolic responses has provided significant insights into the complex interplay between genetics, environment, and obesity. Furthermore, the nematode model has been instrumental in evaluating potential therapies for obesity, helping to identify new compounds and treatments aimed at modulating fat storage and energy metabolism [145]. Studying *C. elegans* is critical for improving our understanding of obesity and its intricacies, paving the way for new approaches to combating this rising public health concern.

9. *C. elegans* in Neurodegenerative Diseases

Neurodegenerative disorders (NDs) refer to a group of age-related diseases characterized by the breakdown of cellular proteostasis and the misfolding of various proteins, leading to the formation of toxic neuronal aggregates [156,157]. In *C. elegans*, ND models are commonly created by overexpressing pathogenic proteins in specific cells (such as neurons and body wall cells) using different promoters [28]. As a result, various transgenic models of NDs have been developed in *C. elegans*. The response of the IGF-1 signaling

pathway to both biotic and abiotic stresses presents valuable insight into the connections between different neurodegenerative diseases. The Alzheimer model of *C. elegans*, which expresses Alpha/Beta-amyloid protein aggregates in the body wall muscle, is paralyzed and reaches smaller sizes than normal. IGF-1, in turn, through the activation of DAF 16/FOXO and HSF-1, reduces these symptoms and induces autophagic clearance of protein aggregates [157]. Interestingly, when the activity of Sir 2.1 is increased, inflammation is reduced, followed by a significant lifespan expansion.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that gradually impairs memory, cognitive abilities, and the capacity to carry out even the easiest tasks [158]. It is characterized by two key neuropathological features: the presence of intraneuronal neurofibrillary tangles, which are associated with neuronal destruction and death in brain regions such as the cerebral cortex and hippocampus, and the accumulation of extracellular amyloid plaques, also known as senile plaques [159,160]. The accumulation of amyloid precursor protein (APP) cleavage products called amyloid plaques results from the insoluble buildup of beta-amyloid peptide (A β). In mammals, the APP protein family includes three members: APP, APLP1, and APLP2, each containing a single transmembrane domain and playing a crucial role in brain development [161,162]. In *C. elegans*, there is a single APP-related gene, *APL-1*, which is exhibited in various organs and is essential for survival [117,146,158].

Notably, *C. elegans* does not produce beta-amyloid peptides naturally because its *APL-1* gene lacks an A β sequence and the β -secretase enzyme (BACE). To mimic the characteristics of Alzheimer's disease (AD), including A β peptide toxicity, several transgenic *C. elegans* strains that express human pathological proteins in muscle cells or neurons have been developed [145,157,161]. Additionally, transgenic *C. elegans* lines replicate the clinical profile of APOE polymorphism-associated neurodegeneration, which provides a valuable model for uncovering novel molecular insights into AD [28]. Several acetylcholinesterase (AChE) inhibitors, such as rivastigmine, donepezil, and galantamine, have been developed to restore normal cholinergic function and support synaptic transmission in the CNS of AD patients. However, the effectiveness of these treatments is limited, with various adverse effects, including gastrointestinal issues [162].

The tau protein homolog in *C. elegans*, called Protein with Tau-like Repeats-1 (*ptl-1*), plays a key role in neuronal health. A loss-of-function mutant of *ptl-1* showed normal development but produced fewer offspring and exhibited reduced touch sensitivity. Additionally, *PTL-1/tau* impacts neuronal integrity and influences the lifespan of *C. elegans* [140,157]. These findings have enabled the development of transgenic *C. elegans* models that accurately reflect tau-related changes seen in Alzheimer's disease (AD). In Parkinson's disease (PD), the brain gradually degenerates, leading to the dysfunction of dopaminergic neurons in the substantia nigra pars compacta (nigrostriatal pathway). This region of the basal ganglia is crucial for voluntary motor control. PD is typically marked by symptoms such as bradykinesia (slowness of movement), muscle stiffness, postural instability, and resting tremors [163].

Various *C. elegans* models have shown great promise in uncovering the factors that could prevent, halt, or even reverse Parkinson's disease (PD) symptoms. These models have also helped identify numerous genetic and pharmacological agents that could prevent dopaminergic neurodegeneration. Among the most notable *C. elegans* models for PD are those that focus on alpha-synuclein (α -syn), a protein prone to misfolding and a key pathogenic factor in PD. This has become a major target for scientific research [164–168]. Transgenic *C. elegans* worms expressing either single or multiple copies of normal or disease-associated proteins under neuronal-specific promoters allow for targeted expression in specific neuronal subtypes. The worms' anatomical transparency makes it possible to

co-express fluorescent proteins, enabling the tracking of neurodegeneration as the worms age [167]. Additionally, the fully mapped connectome of *C. elegans* provides detailed insights into how neurodegeneration affects overall organismal health. It also enables the accurate correlation of cell death with behavioral dysfunction or phenotypic changes in vivo [28].

Since *C. elegans* lacks a homolog of *PARK1*, which encodes α -synuclein, transgenic *C. elegans* are created by overexpressing either wild-type or mutant versions of human synuclein in dopaminergic neurons [166]. In addition, several *C. elegans* genes serve as orthologs to human genes linked to PD, such as *PARK2* and *PAKR5-9*, which encode Parkin [125]. Other key *C. elegans* orthologs associated with PD onset and progression include *pdr-1* (an ortholog of the human Parkin gene), *lrk-1* (an ortholog of the human *LRRK2* gene), and *dj-1* (an ortholog of the human *DJ-1* gene, which encodes glyoxalase mutations). Additionally, *pinl-1*, an ortholog of the human *PINK1* protein kinase, has been linked to autosomal recessive, early-onset PD [159,167]. The versatility of *C. elegans*, combined with continuous discoveries from transgenic models such as those for α -synuclein in PD, demonstrates its potential as a research tool. These findings suggest that *C. elegans* can help divulge new conserved modifiers of genetics. Ultimately, these insights may be translatable to human treatments [160,169].

Huntington's disease results from an increasing trinucleotide CAG (cytosine-adenine-guanine) repeat in the huntingtin gene. This leads to the production of an enlarged polyglutamine (polyQ) tract in the N-terminal region of the huntingtin protein (Htt) [170,171]. Although it lacks a homolog of the huntingtin gene, researchers have created various transgenic *C. elegans* models to study Htt aggregation and toxicity for HD research. While the cholinergic hypothesis is widely accepted in Alzheimer's disease research, there is currently no known drug for this condition. Table 3 lists various chemical compounds that have been shown to alleviate certain neurological conditions in *C. elegans*.

Table 3. Compounds with therapeutic effects on *C. elegans* models in neurodegenerative diseases.

SN	Compounds	Class of Drugs	Diseases	Action Mechanisms	Effects	Ref.
1	Cannabidiol (CBD)	nonpsychoactive phytocannabinoid	AD	It employs an antioxidative mechanism reliant on the classical transcription factors DAF-16 and SKN-1.	Reduces ROS in vivo, but does not overexpress genes related to anti-oxidation.	[169]
2	Cannabidiol (CBD) and dihydroartemisinin (DHA)	Drug–drug conjugate	AD	Conjugates suppressed NF- κ B activation but not MAPK activation in BV-2 cells, hence preventing LPS-induced neuroinflammation.	They exhibit cytotoxicity, significantly limiting their therapeutic windows. The CBD-DHA conjugate C3D reduced neurotoxicity while maintaining anti-neuroinflammation action.	[124]
3	Dihydroartemisinin (DHA)	Artemisinin	AD		Artemisinin alleviates neuroinflammatory responses in the central nervous system (CNS).	[124]
4	Sorafenib	A Raf Kinase inhibitor	PD	Inhibition of leucine-rich repeat kinase 2 (LRRK2).	Increased survival and reduced dopaminergic neuron degeneration in G2019S-LRRK2 transgenic <i>C. elegans</i> .	[171]

Table 3. Cont.

SN	Compounds	Class of Drugs	Diseases	Action Mechanisms	Effects	Ref.
5	GW5074	an indoline compound	PD	Strongly suppresses the autophosphorylation of LRRK2 and G2019S LRRK2.	Enhanced survival and decreased degeneration of dopaminergic neurons in G2019S-LRRK2 transgenic <i>C. elegans</i> .	[171]
6	Rifampicin	Glycation inhibitor		RIF plays a double role by reducing glycation in vivo while simultaneously activating pro-longevity processes through DAF-16.	Extends the lifespan of <i>C. elegans</i> by up to 60%.	[172]
7	Fluoxetine	Selective serotonin reuptake inhibitor	Antidepressant, AD	Possesses antioxidative properties while targeting the Nrf2 signaling pathway. It also exhibits anti-inflammatory/antioxidant actions via inhibiting TLR4/NLRP3 inflammasome signaling pathways.	Resulted in improved antioxidant defense mechanism, decreases in AChE activity, amyloid, Tau protein, MDA, TNF-, and IL1 contents. It also showed cardioprotective benefits by improving lipid profile and significantly lowering cardiac enzymes such as CK-MB, troponin, and MEF2.	[171]
8	Galantamine, haemanthidine	A benzylphenethylamine alkaloid	Moderate dementia (AD)	It is a cholinesterase inhibitor with a dual-action mechanism. As a reversible acetylcholinesterase inhibitor, it enhances acetylcholine's action on nicotinic receptors, improving cholinergic neurotransmission in the CNS.	Delayed paralysis and improved lifespan of transgenic worms.	[173]
9	DMSO	A universal solvent	A β toxicity	Both the presynaptic acetylcholinesterase inhibitor (AChEi) aldicarb and the postsynaptic nicotinic acetylcholine receptor (nAChR) agonist levamisole induced paralysis in <i>CL4176</i> worms, with approximately 70% paralysis observed. DMSO (2%) delayed acetylcholinesterase neurotransmission and appeared to alter Ca ²⁺ ion permeability, which is essential for neurotransmission. Interestingly, both DMSO and ethylenediaminetetraacetic acid (EDTA) provided similar resistance to aldicarb, whether used separately or together.	Delayed paralysis by 48% and 90%, and provided protective effects against A β toxicity.	[174]

Table 3. Cont.

SN	Compounds	Class of Drugs	Diseases	Action Mechanisms	Effects	Ref.
10	Swertiamarin	A secoiridoidal glycoside	Cholinergic dysfunction	It offers neuroprotection by modulating ROS scavenging genes and enhancing cholinergic neurotransmission through the regulation of synaptic AChE.	Amelioration of cholinergic dysfunction.	[175]
11	Glycyrrhizic acid (GA), glycyrrhetinic acid (GRA), liquiritigenin (LG), and isoliquiritigenin (ILG)	Compounds from traditional Chinese medicine (the most active plant for the subject of study) <i>G. uralensis</i>	AD biomarkers, A β aggregates and oxidative damage	These substances may activate the transcription factor DAF-16.	Reduced A β accumulation. It also had positive effects in counteracting protein aggregation and oxidative stress.	[176]

10. *C. elegans* in the Study of Gut Health Permeability

The human gastrointestinal system and that of *C. elegans* share a similar developmental pattern, with the *C. elegans* GI often resembling that of humans [177]. The *C. elegans* gut has microvilli. When observed through an electron microscope, it appears similar to the crypt and villus structures present in mammalian tissue. Its GI system performs essential functions such as nutrient absorption, content transport, and waste elimination. Digestion starts with the pharyngeal grinder, which physically breaks down bacteria, the primary food source for the worm [178,179]. A network of intestinal transporters regulates the balance between nutrients and pathogens. Finally, a rhythmic defecation cycle, driven by calcium ion fluctuations, ensures proper waste elimination [180].

These characteristics make it an excellent representation for studying the gut microbiome. The worm hosts a unique and diverse microbiome, consisting of 12 bacterial species from nine different families, which form the core microbiome based on intestinal colonization studies [181,182]. In the wild, *C. elegans* recruits its gut microbiome from the surrounding environment [183]. Given its bacterial diet, *C. elegans* has adequately represented host–microbiota interactions in mammals [184].

Interactions between gut bacteria and their hosts have been associated with the onset of various human diseases, though the precise mechanisms remain largely unknown. The nematode *C. elegans*, which is genetically tractable, serves as a valuable model for studying gut health interactions, as harmful bacteria such as the human gut commensal *Enterococcus faecalis* can be introduced through feeding. Sim and Hibberd [181] conducted a study demonstrating that infection with *E. faecalis* (along with other potentially pathogenic bacteria) reduced the lifespan of *C. elegans*. An RNAi screen of 17 candidate genes identified the transcription factor *nhr-49*, a crucial regulator of fat metabolism, as responsible for this effect when knocked down. Notably, the similarity between *nhr-49* and mammalian peroxisome proliferator-activated receptors (PPARs) suggests that fatty acid metabolism and innate immunity are involved in this process. *C. elegans* is a bacterivore susceptible to infection through feeding; it represents an excellent system for exploring gut bacteria interactions and pathogenic host–microbiome relationships [183,184].

Graphene oxide (GO) has garnered significant attention for its applicability in biological systems. However, its toxicity is a concern in both biomedical uses and non-biomedical products exposed to the environment. Therefore, understanding its action mechanisms is crucial to identifying potential therapeutic targets in appropriate animal models. A study

by Ren et al. [184] suggests that GO toxicity in *C. elegans* may result from a combination of oxidative stress in the intestinal barrier, increased permeability of the biological barrier, and reduced defecation behavior.

A gap in the intestinal junction barrier is referred to as a leaky gut. When this essential barrier is breached, it causes increased permeability in the gut, allowing big molecules that would usually be unable to cross the gut barrier to seep through and enter the circulation [185]. Models such as pigs, horses, zebrafish, mice, and birds are used for leaky gut assays. However, some of these models are expensive and difficult to maintain. Chickens are susceptible to pathogenic invasion, which could alter research outcomes. Moreover, some biomarkers related to inflammation in mice have been changed due to the absence of specificity in some ELISA isoforms, hence the need for transgenic mouse models, which comes at an extra cost [186,187].

The possession of a worm cell–cell adhesion complex (the apical junction), which has a single electron-dense area, LET-413 that does not colocalize with DLG-1, and the tubular organ membranes' proteins, which include *AR-3*, *PAR-6*, *PKC-3*, and *CRB-3*, are some of the several ways in which *C. elegans* differs from mammals, vertebrates, and drosophila. However, investigations have discovered that *C. elegans* epithelial cells tend to follow similar pathways as human cells, making them excellent for simulating microbial dysbiosis, gastrointestinal inflammation, and disease pathogenesis in the intestine [188]. Indeed, studies have shown that successful treatment techniques for lowering intestinal inflammation in *C. elegans* may be translated into mouse models [189]. This suggests that, despite their simplified anatomy, therapies obtained from *C. elegans* (Table 4) investigations may be used in more complicated mammalian models. Nevertheless, the absence of well-developed organs and systems is a major challenge and could be overcome by combining *C. elegans* studies with other more sophisticated models.

Table 4. Compounds with therapeutic effects on *C. elegans* models in gut health and intestinal permeability related diseases.

S/N	Compound	Drug Class	Action Mechanism	Effect	Ref.
1	Schisandrin C	A dibenzocyclooctadiene lignan	Schisandrin C improved intestinal permeability dysfunction and suppressed NF- κ B and p38 MAPK signaling in Caco-2 cells, which regulate MLCK expression and the structural remodeling of the Tight Junction complex. In both intestinal organoids and <i>C. elegans</i> , Schisandrin C significantly improved aberrant FITC–dextran permeabilization.	Schisandrin C reduces abnormal intestinal permeability and regulates the production of tight junction (TJ) proteins, along with MLCK, p-MLC, and inflammation-related proteins, all of which are associated with leaky gut symptoms and the development of IBD. As a result, it shows potential as a treatment for leaky gut syndrome.	[188]
2	Lipoteichoic acid	Extract from the probiotic organism <i>Lactobacillus paracasei</i> strain D3-5	Regulates the TLR-2/p38-MAPK/NF- κ B pathway.	Displays strong activity to stimulate mucin production and lower aging-related leaky gut and inflammation	[190]
3	Lipoteichoic acid	Lipoteichoic acid from a heat-killed <i>Lactobacillus</i> strain	-	The lipoteichoic acid (LTA) element stimulates mucin production through the TLR-2/p38-MAPK/NF- κ B pathway, strengthening the reduction of age-related intestinal permeability and inflammation.	[166]

Table 4. Cont.

S/N	Compound	Drug Class	Action Mechanism	Effect	Ref.
4	Biacalin	Flavone glycoside		Baicalin (10, 100 mol/L) increased survival and restored behavioral abnormalities in LPS-stimulated <i>C. elegans</i> . It also reduced oxidative stress caused by LPS damage by lowering MDA levels and enhancing SOD activity.	[188]
5	Probiotic cocktail containing five <i>Lactobacillus</i> and five <i>Enterococcus</i> strains	Probiotics derived from human-origin sources	Probiotics modified the microbiota in a way that enhanced bile salt hydrolase activity, increasing taurine levels in the gut, which promoted tight junction formation and reduced intestinal permeability.	Probiotic-modulated gut microbiota primarily alleviated leaky gut by enhancing tight junctions, which subsequently reduced inflammation. Taurine restored intestinal integrity and improved health markers in <i>C. elegans</i> .	[190]
6	<i>E. coli</i> Nissle 1917	A probiotic	Enteropathogenic <i>E. coli</i> (EPEC) infection reduced the lifespan of <i>C. elegans</i> , but pretreatment with the probiotic <i>E. coli</i> Nissle 1917 (EcN) significantly enhanced the survival of worms infected with EPEC.	Increased the expression of tight junction proteins such as ZO-1 in <i>C. elegans</i> , suggesting that EcN enhances gut barrier integrity.	[191]
7	3,3'-Diindolylmethane (DIM)	A digestive byproduct	DIM significantly improved the intestinal permeability of differentiated Caco-2 cells by increasing the expression of tight junction proteins, such as occludin and ZO-1.	It shows potential as a nutraceutical candidate for treating leaky gut syndrome by enhancing intestinal permeability.	[192]
8	Neuronal peptide	Neuropeptigenic regulators	Neuron-specific knockdown of CAPS and PC2 decreased intestinal leakiness occurring due to age, and extended lifespan in <i>C. elegans</i> and <i>D. melanogaster</i> .	Neuronal knockdowns of insulin-like peptides daf-28, ins-7, dilp2, dilp3, and dilp5 antagonized intestinal barrier integrity and lifespan. Furthermore, it reduces insulin signaling in the intestine through the knockdown of the insulin receptor.	[193]

11. Limitations of Using *C. elegans* Models in Pharmaceutical Research

Despite the apparent benefits of utilizing *C. elegans* for drug screening, several factors need consideration. *C. elegans* has a thick cuticle, which often interferes with drug absorption. According to some research, the internal concentration of the tested substance might be less than half of the external concentration, meaning that certain medications cannot cross the cuticle barrier and are thereby dismissed [194,195]. Using *E. coli* as the primary food source has added complexity to the issue. Due to the chemical metabolism or degradation by the bacteria, the use of live bacteria may reduce the effective dosage delivered to the worms. Therefore, higher initial chemical concentrations ranging from (25–100 M) are often employed in *C. elegans*-based pharmacological screening for these reasons [45,195]. Even so, a newly published prospective solution is the use of *E. coli* killed with paraformaldehyde, on which *C. elegans* grows normally and, interestingly, has a normal (non-extended) lifetime despite infection abrogation [196]. Another significant issue is that *E. coli* may biotransform the medication before it reaches the worm. However,

these effects could offer a valuable model for studying how the microbiome influences drug action [196].

Recent research has explored the complex relationships between medications and the bacterial food source of nematodes, as well as the challenge of drugs not being effectively absorbed into nematode tissues. Liposome-mediated delivery has been suggested as a potential solution to address some of the limitations associated with using *C. elegans* as a model for discovering and evaluating anti-aging medicines [195]. The study found that liposome encapsulation positively impacted lifespan extension, reduced the number of compounds needed for analysis, and enhanced the uptake of several dyes into the intestine. However, Texas Red did not penetrate nematode tissues, indicating that liposomes cannot guarantee the pickup of substances [195]. Liposome encapsulation should help minimize drug effects on *E. coli* and drug bio-transformation, often caused by *E. coli*. [196].

Drugs need to reach a specific concentration at their site of action to be effective, and some compounds require much higher levels to have a biological effect. To address this challenge, mutant *C. elegans* strains with wounded cuticles upscale drug absorption [195]. Additionally, the simple anatomy of *C. elegans*, which lacks human organs such as the lungs, liver, kidneys, and blood transport systems, limits its use as a model for studying human disorders linked to these organs. Furthermore, its miniature size can make experimental manipulation difficult for inexperienced researchers [84,94].

While *C. elegans* is an excellent model for studying fundamental biological processes, it is not well-suited for investigating burn wounds or most infections that involve complex tissue repair, immune responses, and systemic interactions [197]. However, recent research on the structure and physiological roles of the *C. elegans* cuticle has provided new insights, highlighting its potential as a valuable model for skin-related studies. This is due to the unique arrangement and organization of collagen proteins in its cuticle [198–200].

C. elegans may facilitate the understanding of associated immune response, host–pathogen interactions, and genetic susceptibility. However, factors including limited infection models involving skin or internal organs, lack of complex host–microbiome interaction, and different pathogen responses are common limitations to its use. For such studies, mammalian models are preferred due to their advanced tissue structures [200].

Model Variation and Reproducibility

Validation entails ensuring that experimental findings in *C. elegans* adequately reflect the biological processes they want to describe. This procedure frequently involves cross-species comparisons and the use of computational tools. Research has shown that toxicity ratings in *C. elegans* correlate well with those in mouse models. For example, studies examining the effects of various metal salts discovered that toxicity orders based on *C. elegans* adult mortality coincided with rat and mouse oral LD50 rankings [201]. Interestingly, developing software frameworks like open worm stimulation stack enables the rigorous, data-driven evaluation of computational models in *C. elegans*. These technologies enable researchers to continuously compare model behavior with experimental data, thereby improving simulation accuracy [202].

Validation of *C. elegans* screening models varies across studies, with some providing strong statistical measures while others offer limited validation details. For example, Moy et al. [134] reported a Z'-factor of 0.61 and a high correlation ($R^2 = 0.98$) with manual scoring, while Rajamuthiah et al. [139] achieved a Z'-factor of 0.77 and validated their results against an agar-based assay. In contrast, other studies provided less comprehensive validation. Kong et al. [137] compared liquid-based and agar-based methods but lacked specific validation metrics, and Moy et al. [138] reported false-positive and false-negative rates without detailed reproducibility data.

Reproducibility is a key component of scientific research, ensuring that results are consistent and dependable. In *C. elegans* investigations, various factors influence reproducibility: A study of over 5000 wild-type *C. elegans* found high inherent variability in lifespan measures, even under controlled settings [203]. This variability shows that finding modest but meaningful effects necessitates bigger sample sizes than are often employed. Also, minor changes in environmental variables, such as temperature, nutrition availability, or salt concentration, can cause adaptive responses in *C. elegans*, drastically affecting experimental outcomes. As a result, stringent adherence to defined cultural practices and handling methods is required to reduce variability [203,204]. Differences in experimental techniques between laboratories may also produce conflicting results. Advocating for consistent reporting and methodological transparency can help to reduce inter-lab variability and increase reproducibility in *C. elegans* research [205].

12. *C. elegans* and Toxicity Issues: A Comparison with Mammals

Drugs are particularly harmful products, and as a result, they must undergo a stringent examination procedure before they can be made commercially available. A major case study of a previous episode is the case of Thalidomide [204]. Consequently, all drugs must be safe, or at least, the benefits must always outweigh the risks [205]. Unlike other products, drug safety cannot be characterized as the complete elimination of risk, as achieving this objective is generally unfeasible within this context. Instead, a drug is deemed fit for use when its potential dangers are manageable, based on an evaluation of its anticipated benefits and available treatment options [204]. Preclinical animal studies are done to elucidate the way they act, describe the proof of concept, and determine how safe new medications are.

Most research, particularly safety assessments, is conducted following worldwide regulatory guidelines. However, animal models used to justify the start of clinical studies have limitations [206], coupled with several regulatory limits associated with their usage that stem from ethical and environmental issues. Since there are no regulatory restrictions on *C. elegans* research, unlike other animal models, developmental, reproductive, and acute toxicity can be assessed in a single experimental design. Additionally, chronic toxicity can be observed as the worms age. The toxicity ranking of various substances in *C. elegans* closely aligns with results from mammals, even though the exact quantities may not be directly comparable [205–208]. Thus, *C. elegans* trials can offer early indications of potential safety risks associated with drugs and nutraceuticals. More interestingly, acute reactions are sensitive probes for evaluating the biological connectivity of the worms to pharmacological treatment, and these connectivities can help to adequately elucidate the activation of previously unknown metabolic pathways both in drugs and in nutraceutical development.

The literature on ethical principles and regulatory frameworks that regulate *C. elegans* in preclinical drug screening processes was quite scarce. However, van der Voet et al. [208] provided 30 reporting criteria for Developmental and Reproductive Toxicology (DART) testing in *C. elegans*. These standards are consistent with the 3Rs principles (Reduction, Refinement, and Replacement) of animal testing and seek to standardize non-vertebrate organism testing for regulatory hazard assessment. The proposed criteria prioritize the documentation of experimental details such as compound purity, lot/batch number, and container type. The study's authors developed these criteria to address reproducibility in *C. elegans* investigations. Their analysis discovered insufficient documentation of ethical principles and regulatory frameworks related to *C. elegans* use in preclinical drug screening, suggesting that current research techniques differ, and existing regulatory frameworks may not adequately cover *C. elegans* models. Additional studies may assist in defining standards for using *C. elegans* in preclinical drug-screening methods.

13. Conclusions

Despite widespread use of various *C. elegans* mutant worms to unravel various disease conditions and drug development processes, it is still difficult to genetically manipulate worms that can accurately mimic pathological conditions of diseases, especially their onset and progression. Also, there is a clear difference between metabolic and nutrient requirements between humans and *C. elegans*, thereby reducing the accuracy of what has been shown in the worms. However, *C. elegans* continues to provide basic and mechanistic insights into biological processes related to the development of pharmaceuticals and nutraceuticals; this is a springboard for further clinical investigations and applications, and a correct appraisal of its knowledge base through standardized and quantitative assays has great potential to speed up the progress of pharmaceutical and nutraceutical research.

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