Journal of Biomedical Research & Studies
Volume 1 | Issue 1

Review Article Open Access

A Systematic Review *Caenorhabditis Elegans (C. Elegans)*-A Host Model Organism to Study Drug-Induced Responses to the Effects of Stimulant and Depressant Drugs

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Citation: Khanh ND (2018) A Systematic Review *Caenorhabditis Elegans (C. Elegans)* - A Host Model Organism to Study Drug-Induced Responses to the Effects of Stimulant and Depressant Drugs. J Biomed Res Stud 1(1): 101

Received Date: May 27, 2018 **Accepted Date**: August 08, 2018 **Published Date:** August 10, 2018

Abstract

Although there are many researches on drug of abuse behavior using *C. elegans* as model organism, the precise targets and mechanism shared by *C. elegans* accompanied with behavioral responses to substance misuse is still scarce. This alternative model for rodents and other animal organism has been used to investigate genetic mechanism and specific genes coding drug-induced behavioral response. However, there is very few papers reported to summarize and analyze findings from original researches to identify target/genes underpinning responses to drug addiction. The consistence in finding of dopamine system involvement in numerous classes of addictive substances helps support a preserved and vital role for dopamine in the drug effects of abuse in animals. This review summarizes and analyzes relevant original research findings to study the effect of stimulant as well as depressant drugs of abuse on molecular and behavioral aspects of *C. elegans*. It helps novices and experts with knowledge base, references, reduce their time and cost for study in this field. Since then, they can further propose in-depth research direction to apply in higher animal, develop treatments/medication for drug of abuse in human.

Keywords: *C. elegans;* Whole Model Organism; Drug-Induced Responses; Effect of Stimulant Drug; Effect of Depressant Drug

Introduction

Drug of abuse triggers many social and economic issues around the world. It has taken a massive toll in financial costs and cause increase in death rate annually with human substance-abuse-related health diseases, cancer, accidents, addiction-associated homicides, violence, etc…. Researches indicate that nicotine and/or cocaine addiction together with long-term alcohol misuse increase mortality in users. The society suffers the heavy drug-abuse-related consequences, estimated of hundreds of billions of USD worldwide. The costs spend for hospitalizations, drug enforcement, healthcare treatments for drug of abuse. Obviously, there is an imperative requirement for alternative and effective treatments and prevention strategies for drug of abuse with understanding of the basic mechanisms underlying abusing behavior. Most of understanding and knowledge people gain about the neurobiology of abuse has been revealed and improved via the use of animal models for many decades [1]. From that the development of behavioral measures and the basic reward circuitry have been discovered and characterized to advance research in human addiction [2]. Many interventions have been applied for detoxification include psychologically behavior therapies including cognitive behavioral therapy (CBT), family therapy and motivational interviewing; and medications to relieve symptoms of addiction, reduce the risk of recurrence, reduce the craving and anxiety of the addicts. In the adolescent and youth group, CBT is applied and considered as a method of success because it helps social skills to be developed, proper interpersonal skills as well as emotional regulatory skills [3-5]. It was stated, communal skills trained complementary to inpatient dealing for alcoholism is likely to be effective, including social environmental management. However, there is a need of combination between psychological therapy and medication to achieve result in treatments. There are a variety of anti-addiction and anti-alcohol medications available on the market including naltrexone, disulfiram , buprenorphine and methadone , but these drugs have side effects and sometimes after using them, the addicts turn to addiction to the drug itself. Those treatments carry the risk of causing physical and or psychological dependence.

On the other hand, the addict faces many other symptoms such as vomiting, nausea, dizziness, headache, cognitive and neural inhibition, memory loss, dry mouth, male ejaculatory difficulty during treatment. Some medications need to perform dual diagnostics to have operative effects on the treatment [6]. Therefore, in summary, the problem is that there must be another direction of research, at molecular level to promote effective medication for human drug of abuse effects. *C. elegans* a temperate-soil worm have been discovered and used widely as a model organism for human disease mechanism at molecular and genetic level. It is considered as the first completely-sequenced-genome animal (with 302 neurons and 959 somatic cells in an adult hermaphrodite approximately 38% of 20.250 protein-coding genes functional homologous to human [7,8]. This is a recent development in study psychiatric disorders such as addiction when using the nematode replacing rodent model which have been used for years. Up-to-date, there are a few available model organism have been characterized and developed to investigate the reinforcing traits of addictive substances. Nevertheless, materialistic evidences showed that this worm is an outstanding host organism to study genetic mechanisms which underpinning the drug effects as well as specific genes/targets for medication or intervention development for those addictive drugs [9]. So far, studies in this field have investigated and measured chemotaxis behaviors, locomotion, egg laying, defecation, body bend amplitude [10-12]. Those quantitated behaviors showing the interaction between *C. elegans* with its environment had been researched for years also the underpinned neuro-biological circuits and systems have been studied and reported, strengthen the role and utility of *C. elegans* as an alternative model system to investigate drug of abuse.

In this systematic review, we will discuss a relatively behavioral measure using *C. elegans* to study drug of abuse. In fact, review papers hole unique place in research worldwide at every level. They help procedure the base for emerging research guidelines, support useful knowledge, trouble shoots in previous researches hence suggest for future research struggles. Among fourteen types of review, systematic review has recently been used and contributed to the research field, especially in human healthcare [13,14]. Remarkably, there is no consensus and agreement about what are *C. elegans* characteristics that would make it a reliable and valid model of drug abuse as well as very few papers reported to summarize and analyze findings from original researches to identify target/genes underpinning responses to drug addiction. This project aims to gather most research findings to build up a systematic review with explicit and methodical schemes to lessen bias in steps of documentation, collection, analysis and generalization of the included studies. Using systematic methods, this review project supports clearly stated research objectives, reproducible methodology, eligibility criteria for included study selection, assessment of the included study validity. Before going to deeper discussion about *C. elegans* model for drug addiction, general context of research field using other animal organism modeling will be relatively provided and discussed in both vertebrates and invertebrates. Following, the uses of *C. elegans* as host model to study other human neurodegenerative disease such as Parkinson's disease, Polyglutamine disease, and Alzheimer's disease [15-17]. Next, some commonly addiction-induced drugs including nicotine, alcohol, cocaine, methamphetamine and amphetamine, their effects in the nematode and various biological systems and molecular targets will be further discussed. Further then, there will be some suggestions to indicate how *C. elegans* model can be refined and developed in future research aspect to make it becomes more validated and more utilized for medication developments, and improve model value for translational applications.

C. elegans - A Model Organism

C. elegans was firstly introduced as a genetically host model by Sydney Brenner in 1965, then it has been widely used in various fields of study including aging and diet innate immunity neuroscience developmental biology and ecotoxicology [7,18-22]. This non-parasitic nematode can be found in rotting fruit, compost heaps, snails and mostly in the temperate soil [111]. Since the nematode has been used in various fields of research, there are a number of breakthroughs in biomedical science have been developed such as the use of the protein marker from green fluorescent proteins, the discovery of RNA interference (RNAi) and genetic regulators of programmed cell death [22]. The worm has anatomical and genetic characteristics that make it becomes a good model organism: (i) small size (approximately 1 to 1.5mm in length and 80μm in diameter), (ii) short reproductive cycle, (iii) short lifespan, (iv) translucent body, (v) precise and predetermined anatomy, (vi) easy to culture in large number, (vii) small genome, (viii) whole genome sequenced, (ix) RNAi library available, (x) deletion mutant database [22]. Furthermore, those characteristics strengthen the invertebrate to be an easy experimental model to study biological process with low costs and time-consuming. To be more specific, temperate soil worm is long 1 to 1.5mm, use different bacteria, mainly *Escherichia coli* as their food source [7]. This worm with two sexual forms, male and/or hermaphrodite, is self-fertilize, and able to produce eggs and sperm by its own when it gets mature stage. In natural environment, the males are normally with very low ratio (about 0.02% of the nematodes), however the percentage in their offspring may be multiplied about 50% in the hermaphrodites among themselves [23]. Favorable temperature for the worm to grow ranges from 16 to 250C which is normally used in laboratory. Wild type N2 worm strains will have life cycle and their lifespan depending on the growth temperature [22]. In 200C temperature, an adult worm will normally lay 300 to 350 eggs which will hatch to reach the adult stage after the larva stage within three days. The nematode has estimated lifespan varies from 18 to 20 days under suitable living conditions [16,24]. Remarkably, their life cycle and lifespan are inversely proportional to the temperature [22]. Meaning, the higher the temperature in the worm living environment, the shorter their lifespan and their life cycle will be. The worm was predetermined and well-dissected anatomy. The adult worm has precisely 302 neurons and 959 somatic cells [7,25, 26]. Their transparent body allows for observation of cell fate determination during development, expression of florescent tagged proteins of interest, the genetic regulation of life span, the mechanism of RNA interference, the process of apoptosis. This worm is firstly well-genome-sequenced species and loss-of-function mutants for the majority of genes are available from public databases and resources [20]. A report by Markaki and Tavernarakis in 2010 stated that approximately 42% of the target genes caused for human diseases are homologous to which in the worm [27].

C. elegans - An Organism Homologous to Human

There are several scientific breakthroughs demonstrated that many proteins genetically preserved between *C. elegans* and human [22]. From the success of sequencing of the worm, since then genetic screening techniques have been used and the scientists found that about 36% of 18.891 *C. elegans* proteins have homologs to that of human (set of 4.979 proteins sequences) by pairwise comparison [28]. Then this percentage was identified increased up to 83% in study of Lai and his colleges in 2000 [29]. In 2011, Shaye and Greenwald conducted a study and generated a compendium of *C. elegans* genes (composed of 7.663 protein-coding genes) with human orthologs. It was estimated 38% of the 20.250 gene coding for the worm has unique corresponding functional orthologs in human genome. To be concluded, insight in biological processes in the *C. elegans* can support scientists in human biological research and the orthologs between human and nematode genes strengthen that this is a useful host model for molecular and genetic researches associated with human diseases to develop new therapeutic intervention against human diseases [8,27].

C. elegans - A Model to Study Drug of Abuse

C. elegans becomes a popular superior for study of drug of abuse instead of other animal model organisms including primates other mammals, rodents [30-35]. Although throughout the years these models have been widely used for addiction research based on their structurally and functionally basic neurobiological systems with human, comparatively little understanding in molecular foundations and mechanism of substance misuse has yet to be developed. More recent works indicated that many no-genetic and genetic mechanism underlying drug abuses are present in invertebrates including crayfish, *Drosophila melanogaster* and *C. elegans* [36-38]. The nematode with conserved neurobiological systems to that in human is an obvious choice modeling to study various disease states [38]. Invertebrate models provide genetic and molecular tools (completely genome sequenced thousands of deletion mutant databases, gene manipulation and expression through RNAi techniques and transgenic approaches, short life cycle with lower cost as compared to higher organism models. To date, many publications used *C. elegans* as a model to study various diseases and disorder in human including neurological diseases, mechanisms underlying neurodevelopmental disorders and neurodegenerative diseases [38-41]. A relative recent development is the usage of the worm as a model to investigate drug of abuse and or psychiatric disorders [30-33]. Like mammals and other invertebrates, it has been discovered that the *C. elegans* shows conditioned preference for cues after preceding parings with methamphetamine and cocaine substances, which depends on dopamine neurotransmission [42]. The worm also shows tolerance, cross-tolerance, sensitization and cross-sensitization which are all symptoms appear in human. There are remained limitations including nonexistence of some neurotransmitter systems (norepinephrine or noradrenaline). Likewise, till date the reinforcing properties of substances have limitedly studied and characterized using valid behavioral models. Many studies on the non-genetic mechanisms include defecation, egg-laying, locomotion and postural measures, chemotaxic behaviour nonassociative and associative learning and genetic and molecular mechanism that underpinning the drug-induced responses [11,43,44]. Those behaviors which are underpinned by the circuits and the neurobiological systems have been studied and described for years using *C. elegans* model, indicates that this model is an brilliant experimental model to investigate the drug-induced responses and to develop potential targets for medications or interventions in human drug addiction issues.

Material and Method

Systematic Review and PRISMA-P Protocol

Review papers hole unique place in research worldwide at every level of healthcare system. They help procedure the base for emerging research guidelines, support useful knowledge, trouble shoots in previous researches hence suggest for future research struggles [45]. This information is useful for shareholders across the healthcare facilities. A protocol, a prior plan in conjunction with documentation of a methodical approach makes a systematic review become more rigorous and trustworthy in large scale. There are fourteen types of review paper and systematic review has recently been used and contributed to the research field, especially in human healthcare globally [14,46]. There are remarkable reasons for a systematic review protocol to be important: (i) it allows critics or scientists to systematically plan and thus predict trendy issues; (ii) since then the reviewers can clearly document what is prearranged before they review to allow novices/non-experts can consider the protocol as well as the completed review, to repeat the review process if wanted, and to appraise the validity of planned approaches; (iii) it avoids illogical assessment with deference to enclosure criteria and data extraction procedure; (iv) it may lessen repetition of efforts and increase alliance and partnership in need. Nowadays, international organization including Agency for Healthcare Research and Quality (AHRQ) and the Cochrane and Campbell Collaborations require and publish protocols. Apart from it, other sites and publishers published completed review without working from a planned protocol as well as the availability of excessive duplication and selective reporting of review among authors with the same or related topics is increasing [47]. To overcome these issues, in 2011, organized an international PROSPERO, which stands for Prospective Register for Systematic Review Protocols (see the link www.crd.york.ac.uk/prospero/) over the Centre for Reviews and Dissemination at the University Of York (UK). Since then, with total of from 69 countries, there were >5000 systematic review protocols have been registered systematically. In 2015, the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-analyses) has been built by an international group of experts for improving the completeness, accuracy, transparency and frequency of documented systematic review and meta-analysis protocols—PRISMA-P. There are 17

items with 26 sub-items in total included in the checklist of PRISMA-P. Three main sections in the checklist include administrative information, introduction, and methods [45]. This systematic review follows PRISMA-P protocol aims to summarize knowledge, analyze included research findings (using host model *C. elegans*) with strength and weakness, point out specific target/genes identified underpinning drug-induced behavioral responses through almost drugs of abuse and from that novices and experts may have in-depth research direction to apply in higher animal, develop interventions for drug of abuse issues in human with lesser time and costs.

Eligibility Criteria, Search Strategy, and Studies Included

Eligibility Criteria: The articles used in this systematic review were generated from unpublished (author manuscript) and published research reports. Studies were required to be original researches using the nematode as a molecular and genetic model to investigate drug of abuse. Actually, *C. elegans* is eligible for various approaches, not only in drug of abuse study, but also in drug discovery study and other human diseases. In this review, we focused only on the use of *C. elegans* in genetic and mechanism study of drug of abuse issue. Each of individual research must have enough result with sufficient replications to ensure the outfits are reliable and valuable in this field of study. To be more specific, within each experiment, the treatment conditions compared must involve placebo treatment, no treatment, general practice steadily, or two discrete treatments. Last but not least, all of the included studies in this review must be written in English in 1980 afterward.

Literature Search: The initial search was conducted in September 2017 using a variety of reliable sources including bibliographic electronic databases including Scopus (Elsevier), Sciencedirect (Elsevior), UK Pubmed Central, Web of Science, Web of Knowledge due to their reliability and consistency. Those bibliographic databases were used to conduct the search string [C. elegans] AND [drug of abuse] OR [depen*] [drug] OR [abuse] OR [misuse] OR [addiction]. Another string was also used to search with those key words but with the full name of the *Caenorhabditis elegans*. Hand-searches with no limits in those electronic databases were also conducted using search terms included keywords such as ethanol, nicotine, cocaine and methamphetamine, which are main substance or drug leading to abuse and were mainly focus on drug of abuse studies so far. From the retried studies, potentially eligible studies were reviewed and added into include list of studies for the review. Overall, the search (with the same string) had obtained 257 original studies (out of 1516 total records) in Sciencedirect (Elsevior), 35 original studies (out of 51 total records) in Web of Science, 26 original studies (out of 39 total records) in Scopus (Elsevior). For individual search with specific term in drug issue, ethanol was with total of 139 records (120 original studies), nicotine is with 63 total records (54 original studies), cocaine was with 13 total records (08 original articles), and methamphetamine is with 06 results (05 original studies). All the mentioned articles (both with published and unpublished ones) above were reported from 1980 through 2017. In summary, there were 63 pertinent original studies out of 103 records were investigated and reported in this review.

Selection of Studies: All studies using *C. elegans* as a model organism to study drug of abuse and related issues published in English up to December 2017. Inclusion and exclusion criteria were applied for selection process to narrow down the included studies eligible for this systematic review. Inclusion criteria were: (i) original studies using the worm as a tool for drug addiction and dependence study, (ii) focus on specific substance such as cocaine, nicotine, methamphetamine or ethanol, (iii) were published in peer-reviewed journals, (iv)have been done with experiments with sufficient replication, (v) were reported in English language. Studies were excluded if they: (i) not completely reported in English, (ii) books, book chapters, systematic and/or literature review papers, regular article, index, author index, paper alert, content volume, manual or experimental guidelines, (iii) using the nematode as a whole organism for other direction of research such as neurodegenerative disease or drug discovery, (iv) using other model organisms such as rat, mice, fish, yeast, rodent in their experiments. Mendeley program have been used efficiently in checking and removing duplicated journals out of the list. After that, those which have exclusion criteria are also removed out of the list of eligible studies. A total of 1.667 papers were removed because they are not satisfied for the selection criteria. All remain papers were assessed for inclusion and exclusion criteria and only those meet inclusion criteria were kept for further assessment. Screening process has been done for relevance of identified studies by cautiously looking through their titles and abstracts. Figure 1 delineates current visual aid of literature search and screening process results.

Outcome Variables: The principal outcomes of this systematic review were the effects of drug of abuse, including stimulant and depressant ones. Secondary outcomes were examined including were behavioral (attention, verbal, sexual, and locomotion) mechanism, genetic mechanism (gene expression), plasticity, nervous system function, environmental manipulation, mobility, fertility, reproduction (egg-laying and egg production), lifespan, life expectancy, preference, physical development, neurochemical changes, molecular mechanism, gustatory plasticity. The focused substances examine in this systematic review were ethanol (n=39), nicotine (n=19), cocaine (n=3), amphetamine (n=1) and methamphetamine (n=1) (Figure 1).

Data Extraction Method: A protocol used for data extraction was conducted to extract information and findings from the original studies in this systematic review. Extracted data were authors, year of published, substance in the study, research focus, concentration used, sample size traits, duration of study, sample characteristic, principle finding, strength and limitation. Author carefully read through all selected papers in their entireness three times and using data extraction form to abstract the studies form to ensure the accuracy of extracted data. Then, mutual discussions with colleagues and experts about disagreements of the data in the extracted form were hold to make sure the exactness of the information hauled out from selected studies.

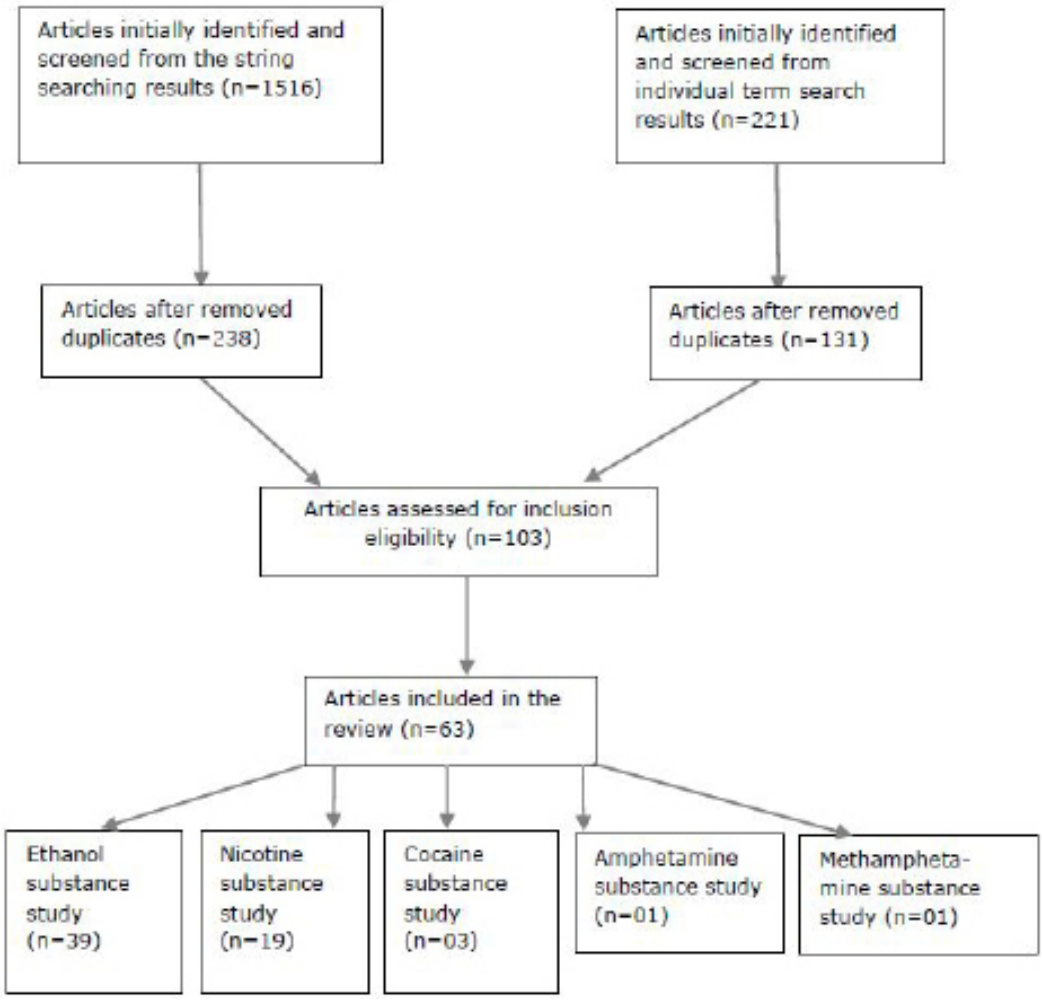

Figure 1: Delineates Current Visual Aid of Literature Search And Screening Process Outcomes

Results and Discussions

Features of Selected Studies

A total of 63 selected studies scanned to study drug-induced responses to effects of stimulant and depressant drugs on the worm *C. elegans*. This systematic review includes 19 studies with nicotine substance, 39 studies with ethanol, 03 studies with cocaine, 01 study with amphetamine, and 01 study with methamphetamine. Of those examined studies were all original researches. Among depressant substances such as opioid morphine, heroin, codeine, anesthetics, benzodiazepines, barbiturate, alcohol was the most examined substance to date (total of 39 studies). For the stimulant substances, including amphetamine, methamphetamine, caffeine, cocaine, nicotine, Khat, nicotine was the most frequently examined (with 19 studies in this review). The remaining studies were cocaine substance (with 03 studies), amphetamine (01 study) and methamphetamine (01 study). There was no study examined the effect of Khat, barbiturate, codeine, heroin, morphine, benzodiazepines on the worm. Table 1 shows the fact that the nematode has been gradually used to study drug-induced responses to the effects of stimulants and depressants year by year with increasing number of publications. Most of the included studies focused on genetic mechanism (88.89%) and locomotion characteristics of the treated worm in their studies. Regarding duration of treatment, due to toxicity and neurotoxicity of the tested substance, most of the experiments the researchers used acute - minutes of exposure (77.79%) rather than chronic exposure - hours of exposure to the worm population. All of the studied discussed theory in their report together with experiments. For ethanol, the concentration of 0-400mM was most commonly used in the test (56.41%). For the stimulants, the concentration of 0-50mM was mostly used in their experiments (58.33%). Most of the studies included examined the genetic mechanism in which the worm responds to the effects of stimulant and depressant drugs. In addition, to be more precise and detailed, the researchers studied behavior, locomotion, egg-laying, sexual response, development, toxicity. There were some studies combined genetic mechanism and one of those aspects such as locomotion or behavior to further evaluate in both phenotypic and genetic changes and responses of the worm to drugs. The dose of treatment varies according to different protocol, ranging from 0 – 1.5M of the examined substance (Table 3). Most of the included studies tested the acute condition of treatment while some others studied chronic exposure of the substance and their effects on the nematode. Out of the included studies examined the effects of drug on the adult worm, there

were 02 ones studied the effects of ethanol on the embryonic state of the worm, they tested physical development, reproduction, life expectancy; 01 paper tested the effects of methamphetamine on the embryonic state of the worm [61,62,94].

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings	Strength	Limitations/ Suggestion	Ref
1	Davies *et al.,* (2003)	Ethanol	Genetic mechanism Locomotion, egg laying	-Dose of treatment: 0- 1OOmM of ethanol -Sample size: 1Ow-orms/test -Duration: 4s -2mins	- Selective activation of *BK* channels is responsible for acute intoxicating effects of ethanol in *C. elegans* -slo-1 has a central role in ethanol responses which encodes the BK potassium channel	-Role of the BK potassium channel in behavioral responses to ethanol in *C. elegans* -Ethanol Resistance by Loss of *slo-1* function in *C. elegans* -Showed a direct rela-tionship between *the SL0-1* protein and a :;?;enetic effect on behavior	No future stu dy suggested by the authors	[48]
2	Mitchell *et al.,*	Alcohol	Behavioural and genetic mechanism	-Dose of treatment: 47, 121, 227 and 363mM of ethanol for the chronic exposure; 50, 100, 250 and 400 mM for the acute exposure -Sample size:,_,5Oworms/ test -Duration: 0-125mins	-The potassium channel, *slo-1*, which is a candidate ethanol effector in *C. elegans*, is not required for the re-sponses described here. -A mutant deficient in neu-ropeptides, *egl-3,* is resistant to withdrawal (al-though it still exhibits acute responses to ethanol). -Involvement of a num-ber of neuropeptides in chronic responses to alcohol: corticotrophin releasing-factor (CRF), opioids, tachy-kinins as well as NPY	-Acute and chronic condition of alcohol ex-posure was examined. -Investigated the genetic basis of ethanol-induced neural plasticity. -Demonstrates the phenomenon of ethanol withdrawal and withdrawal relief in *C. elegans.* -Provide a redu ctionist correlate of ethanol in-duced neural plasticity which underpins nega-tive reinforcement and therefore contributes to alcohol addiction	Future works needed to open the way for a genetic analysis of the ef-fects of alcohol on a simple model system.	[49]
3	Kayser *et al.,* (2003)	Ethanol	Genetic mechanism	-Dose of treatment: 0, 0.25, 0.5, 1 and 1.5 M of ethanol -Sample size: # -Duration: 5 mins	-Ethanol inhibited complex I-, II-, and III dependent oxidative phosphorylation in isolated wild-type mitochon-dria at concentrations that immobilize intact worms -The inhibitory effects of ethanol on mitochondria from either *C. elegans* or rat skeletal muscle were revers-ible even at molar concentra-tions	- Concluded threshold value of complex I activity controls the transition from mobil-ity to immobility of *C. elegans*.	-Do not indicate whether *gas-1* and *complex* I are etha-nol targets. -Do not know what the effects of etha-nol permeability or metabolism might be on the results	[50]
4	Hawkins *et al.,* (2015)	Alcohol	Gene -genetic mechanism Locomotion	-Dose of treatment: 100 to 500 mM ethanol -Sample size: 10 worms/group -Duration: 1, 5, 10, 20, 30, and 40 min	-Cholinergic signal-ling through a specific a subunit-containing *nAChR* is involved in ethanol-induced excitation -Tolerance to this ethanol effect is modulated by Na+/K+ ATPase function. -Identified an excitatory effect of ethanol that we have termed EHC in the model organism *C. elegans* and developed a novel software assisted assay method to aid in data analysis	-Used both genetic and pharmacological ap-proaches to determine the molecular mechanisms involved in this ethanol-induced effect	No future stu dy suggested by the authors	[51]

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings	Strength	Limitations/ Suggestion	Ref
5	Yuan *et al.*, (2008)	Alcohol	Genetic mechanism	Dose of treatment: 50mM of alcohol -Sample size: # -Duration: 0-2-20 mins	Tolerance was observable in *BK* Ca channels in membrane patches pulled from *HEK* cells and when they are placed into reconstituted 1-*palmitoy l-2-oleoyl-sn-glycer o-3-phosphat idylethanolamine/1-pal mitoy l-2- oleoyl-sn-glycer o-3-ph osphatidyl serine* membranes -Tolerance can be an intrinsic property of the channel protein-lipid complex -Bilayer thickness plays an important role in shapin, the pattern of response to ethanol	-Examined two additional aspects of tolerance in human BKCa channels (1) Is acute tolerance observed in a single channel protein complex within a lipid environment reduced to only two lipids? 2) Does lipid bilayer composition affect the appearance of acute tolerance?)	Do not have su fficient data to discriminate among the various mechanistic models including the one-site and two-site possibilities (interactions between the drug and the lipids surrounding the channel protein; multiple direct interactions between alcohol and the protein)	[52]
6	Topper *et al.*, (2014)	Alcohol	Behaviour (attention, verbal, sexual, and locomotor behaviours) Genetic mechanism	-Dose of treatment: 500mM of alcohol -Sample size: 10-15 worms per assay -Duration: 30 mins	-The nematode displays distinct behavioural states associated with locomotion (crawling on land and swimming in water) that are mediated by dopamine -Additional behaviours, including a variety of escape responses are also inhibited in water -Whereas alcohol non-specifically impaired locomotion, feeding, and escape responses in worms on land, alcohol specifically disinhibited these behaviours in worms immersed in water -Loss of dopamine signalling relieved dis inhibition of feeding behaviour, while loss of the *D1-like dopamine* receptor DOP-4 impaired the ethanol-induced disinhibtion of crawling	-Uncover conserved molecular mechanisms that underlie alcohol induced dis-inhibition of behaviors in higher animals. - Provide an excellent model to study dis-inhibition and provide evidence for a role of dopamine in the response to EtOH in C. elegans	-Only one does of treatment was examined -Need more work to examine direct interaction of EtOH on dopmine receptors.	[53]
7	Davies *et al.*, (2015)	Ethanol	Locomotion Genetic mechanism and environmental manipulations	-Dose of treatment: 0- 400 mM of ethanol -Sample size: 10 worms/each test -Duration: 10-12 mins, 30-32 mins of ethanol exposure	-Examine the roles of particular genes and environmental factors in behavioural responses to ethanol, in which locomotion is the behavioural output -Ethanol dose-dependently causes an acute depression of crawling on an agar surface -Animals exposed to a high concentration demonstrate an initial strong depression of crawling -Partially recover locomotion speed despite the continued presence of the drug	Clearly expressed the strength and limitation of the experiment Strength: the use of 10 worms per copper ring + the 10-12 min time window⁺ The settings could be altered -?more advantages	Mutant worms that have very significantly reduced basal speeds, due to incoordination or near paralysis, may be identified as falsely positive for resistance to the effects of ethanol because their measured speed decreases to a value that is below the threshold of accurate detection for a particular imaging setup	[54]

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings	Strength	Limitations/ Suggestion	Ref
8	Yu *et al.*, (2011)	Ethanol	Mobiity, Fertiity, lifespan, sensory response Development	-Dose of treatment: 0%, 2%, and 5% ethanol -Sample size: over 100 worms per plate Duration: 0-40 days (lifespan test) 7days (mobility test) 8 days (development, fertility, sensory response)	-At high concentrations (>=4%), ethanol significantly impaired mobility, reduced fertility, and shortened lifespan -At low concentrations (1-2%), it extended lifespan, accompanied with a slower decline of mobility during aging, although it slightly impaired development, fertility, and chemotaxis	Demonstrated hormetic effects of ethanol and further established *C. elegans* as a suitable animal model to study ethanol related problems	-Did not observe in *age-1* and *sir-2.1* mutant worms. -Further application of the C. elegans model system for stu dying the effects of ethanol on health.	[55]
9	Brodie *et al.*, (2007)	Ethanol	Genetic mechanism	-Dose of treatment: 0.2- 0.5% vIv or 40-100 mM of ethanol -Sample size: Duration: #	-Ethanol directly modulates *BK channel* activity in a variety of systems (*C. elegans* type IV dopaminergic CEP neurons) -*SK channels* modulate ethanol stimulation of neurons that are critical in reward and reinforcement. -*C. elegans slol null* mutants are resistant to ethanol-indu ced motor incoordination	-Indicate that CAK (both SK and BK) channels represent relevant targets in ethanol actions.	No future stu dy suggested by the authors	[56]
10	Bhandari *etal.* (2012)	Ethanol	Genetic mechanism	Dose of treatment: 0- 400mM of ethanol -Sample size: # Duration: 5-10-30 mins	-Conserved role for Chloride Intracellular Channels (*CL/ Cs*) in alcohol related behaviour. - Mutations in two *C. elegans Clic* orthologues, *exc-4* and *exl-1,* altered behavioral responses to acute ethanol in worms, and that viral-mediated overexpression of *Clic4* in mouse brain decreased the sedating properties of ethanol -Demonstrate key roles for *Clic* genes in behavioural responses to acute alcohol in	Spontaneously studied in *C. elegans*, mou se and Drosophila to investigate this and other possible mechanisms of CLIC proteins in behavioral responses to ethanol	-Did not observe altered ethanol metabolism in other AAV gene delivery studies on PFC (prefrontal cortex) -More works needed to investigate whether variance in *Clic* genes might be associated with human responses to alcohol or alcohol abuse	[57]
11	Peltonen *et al.* (2013)	Ethanol	Genetic mechanism	-Dose of treatment: 0.2M of ethanol -Sample size:# Duration: 7 days (for RNA isolation)	- Using RNA-Seq and quantitative real-time PCR. -*Cy tochrome P-450 (CYP)* gene family members (12 of 78) were up-regulated, whereas activated in blocked unfolded protein response (*ABU*) (7 of 15) were down-regulated in chronic ethanol exposure	Provide biochemical and molecular mechanisms of ethanol toxicity that should be useful also in higher organisms.	Only one concentration of ethanol exposure was examined	[58]
12	Davis *et al.* (2014)	Alcohol	Genetic mechanism	-Dose of treatment: 1.5 - 2 mM of ethanol -Sample size: 10-20worms/plates Duration: 5-20 mins	-The *T3521*mutation selectively disrupts ethanol modulation of the BK channel. -The *T3521*mutation may alter a binding site for ethanol and/or interfere with ethanol- induced conformational changes that are critical for behavioural responses to ethanol.	This experimental design is useful for drug targets that cannot be assessed easily at the molecular level	-Did not record from the worm *SL0-1* channel. -More search needed for additional residues in the BK channel important for the actions of ethanol, or residues in other proteins involved in the actions of ethanol or other drugs	[59]

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings	Strength	Limitations/ Suggestion	Ref
13	Davies *et al.* (2012)	Ethanol toluene	Behaviour Locomotion Genetic mechanism	-Dose of treatment: 200- 400mM of ethanol; 0- 12,000 ppm of toluence -Sample size: 10 worms for each test -Duration: 20 mins of ethanol exposure; 10 mins (toluence)	-Ethanol and toluene have distinct behavioral effects and minimal overlap -Mutants of the *slo-1, rab-3* and *unc*-64 genes that are resistant to ethanol -Mutation in the *unc-79* gene results in hypersensitivity to ethanol	Provides a unique and sensitive means of delineating both the commonalities as well as the differences in the neurochemical effects of classical CNS depressants and abu sed volatile inhalants	Future study need to identify the gene(s) that have been mutated in these strains so as to better understand the mechanism of action of toluene	[60]
14	Lin *et al.*, (2013)	Ethanol	Development	-Dose of treatment: 5-10 and 20% EtOH (w/w in ddH20) -Sample size: use embryo of the worm Duration: 8hrs	- The longitudinal effects of EtOH on development using age-appropriate markers and then closely followed embryonic development before, during, and after EtOH exposure -10% EtOH, embryos were at younger embryonic stages, hatched later, and had higher mortality compared to unexposed controls -5% EtOH were at normal embryonic stages, showed no change in mortality, but hatched later than controls -When exposure to EtOH, shorter mean body lengths and slower postembryonic development	- Highlighted the importance of investigating EtOH-induced defects using different markers and at multiple time points. -This is the first study addressing the temporal dynamics of developmental delay during and after EtOH exposure *in vivo*	Should invest more attention on potential treatments during the immediate post-exposure period to discover treatments tailored to the timing relative to the EtOH exposure period	[61]
15	Davis *et al.*, (2008)	Ethanol	-Physical development, -Reproduction -Life expectancy	Dose of treatment: 0.0, 0.1, 0.2, and 0.4 M of ethanol Sample size: # Duration: 0-15 days	-Chronic exposure to ethanol during larval development temporarily delayed physical growth, slowed development, delayed the onset of reproductive maturity, and decreased both reproductive fecundity and longevity -Acute embryonic exposure of *C. elegans* eggs to high concentrations of ethanol at different stages of development resulted in a lower probability of exposed eggs hatching into larval worms depending on when eggs were exposed during development.	-Describe the effects of chronic exposure to ethanol during larval development on *C. elegans* -Test both chronic and acute exposure of ethanol	-Future study need to screen for genes involved in conferring resistance or increased susceptibility to the teratogenic effects of ethanol. -Use forward genetics to identify the mutation that confers either resistance or tolerance to the teratogenic effects of ethanol	[62]
16	Oh *et al.*, (2017)	Alcohol	Genetic mechanism	-Dose of treatment: # -Sample size: > 20 worms/each test, total of 350 worms for the test Duration: 0-250 mins	-The control of *BK* channel trafficking is a critical regulatory mechanism for synaptic transmission and neural function. - *ERG*-28, an endoplasmic reticulum (ER) membrane protein, promotes the trafficking of *SL0*-1 BK channels from the *ER* to the plasma membrane by shielding them from premature degradation.	-Indicates that the control of BK channel trafficking is a critical regulatory mechanism for synaptic transmission and neural function. -Speculate that ERG-28/DDI-1 adjusts overall SL0-1 levels depending on cellular stress or physiological necessity	-Further studies are needed to definitively assess the functional conservation between *C. elegans* and mammalian ERG28 proteins	[63]

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17	Wang *et al.,* (2011)	Ethanol	Genetic mechanism Molecular mechanism Gustatory plasticity Locomotion	-Dose of treatment: 0- 400 mM of ethanol -Sample size: 100-200 worms Duration: #	-Ethanol administration interfered with gustatory plasticity during pre-exposure or test stage in well-fed worms -One mutant previously implicated involved in acute ethanol responses, *slo-1* -Two mutants with defects in serotonin synthesis, *tph-1* and *bas-1*, failed to exhibit ethanol interference with gu statory plasticity. -Two metabotropic serotonin receptors, SER-4 and SER-7, were found to be involved in ethanol-mediated gu statory plasticity -The *tph-1* and ser-4 loci were also involved in ethanol's effect on locomotion behaviour	This study is a novel example of the ethanol effect on associative learning behavior	More works needed to contribute to further understanding of mechanisms underlying ethanol intoxication	[64]
18	Alaimo *et al.* (2012)	Ethanol	Behaviour Genetic mechanism	-Dose of treatment: 0- 500 mM of ethanol -Sample size: Duration: 10 -50 mins of exposure	-Independent inactivation of an ADH-encoding gene (*sodh-1*) or an ALDH-encoding gene (*alh- 6 or alh-13*) increased the ethanol concentration in worms and caused hypersensitivity to the acute sedative effects of ethanol on locomotion. -The sensitivity to the depressive effects of ethanol on locomotion is strongly influenced by the osmolarity of the exogenous ethanol solution.	-Indicate that ethanol metabolism via ADH and ALDH has a statistically discemable but surprisingly minor influence on ethanol sedation and internal ethanol accumulation in worms. -The osmolarity of the medium has a more substantial effect on the observed sensitivity to ethanol.	The authors suggested that both metabolism and environmental conditions should be considered in the analysis of mechanisms that contribute to ethanol responsive behaviors	[65]
19	Lee et al., (2009)	Ethanol	Genetic mechanism Behaviour Chemotaxis Preference	-Dose of treatment: 300 mM of ethanol -Sample size: 100-200 worms Duration: 30 mins of exposure	-Animals show ethanol preference *after 4 h* of pre-exposure to ethanol and exhibit significantly enhanced preference for ethanol after a lifetime of ethanol exposure. -The *cat-2* and *tph-1* mutant animals have defects in the synthetic enzymes for dopamine and serotonin, respectively.	-Designed a behavioural assay for testing ethanol preference after prolonged ethanol exposure. -dopamine is required for ethanol preference.	-Further study need to determine if the ethanol preference defects of *cat-2* and *tph-1* are additive. -Further genetic studies of ethanol preference in *C. elegans* are needed	[66]
20	Patananan *et al.* (2015)	Ethanol	Genetic mechanism Life span	-Dose of treatment: 0- 17-400 mM of ethanol -Sample size: 100-150 worms Duration: 2-3hrs at room temp.	-Lower concentrations of ethanol (0.86 - 68 mM) cause a two- to three-fold *increase in the life span* of animals in the stress resistant L1 larval stage in the absence of a food source -Use biochemical assays and next generation mRNA sequencing to identify genes and biological pathways altered by ethanol -RNA-seq analysis of Ll larvae incubated in the presence of 17 mM ethanol resulted in the significant differential expression of 649 genes, *274* of which were downregulated and 375 were upregulated	Provide insight into not only the longevity pathways in *C. elegans*, but also in those of higher organisms	-Did not observe changes in the expression of these genes in our study using 17 mM ethanol.	[67]

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21	Kwon *et al.* (2004)	Ethanol	Genetic mechanism	Dose of treatment: 7% (v/v) ethanol -Sample size: 50 worms for each spot Duration: 15 and 30 mins of exposure	-Identified *230 genes* affected by ethanol and most of them are heat shock protein. -Two non-heat shock proteins: *glr-2* was the only glutamate receptor gene to be induced by ethanol. *T28C12.4*, which encodes a protein with limited homology to human neuroligin, was also specific to ethanol stress.	Identified a regulatory element, TCTGCGTCTCT, that was necessary for the expression of subsets of ethanol response genes.	No suggestion was stated by the authors	[68]
22	Adskin *et al.*, (2017)	Ethanol	Behaviour Genetic mechanism	-Dose of treatment: 400 mM of ethanol -Sample size: Duration: 10 mins of exposure	-Detected significant association in *COL6A3and* suggestive association in 2 previously implicated loci, *KLF12* and *RYR3* -Knockdown of a *COL6A3* ortholog in - Caenorhabditis elegans reduced EtOH sensitivity. -Loss of function of the *RYR3* ortholog reduced EtOH sensitivity in *C. elegans*	-Have limitation section separately. -Implicate *COL6A3, KLF12, RYR3,* and *LOC339975* in response to EtOH across multiple species and/or AD risk in humans	-The functions of long noncoding RNAs are poorly understood. -Unscreened controls -Lack of strong human replication. -Limited phenotypic consilience	[69]
23	Bettinger et al., (2012)	Ethanol	Genetic mechanism Development	-Dose of treatment: 300 mM of ethanol -Sample size: 10 worms of each group Duration: incubated for 90 mins	-Performed a genetic screen to identify genes required for the development of acute functional tolerance to ethanol in the nematode *C. elegans*. -Genetic manipulation of *lips -7* expression, up or down, produced opposing effects on ethanol sensitivity and on the rate of development of *AFT*. -Decreasing cholesterol levels through environmental manipulation mirrored the effects of decreased *TAG* levels. -Genetic alterations in the levels of the *TAG* lipase *LIPS-7* can modify the phenotype of gain-of-function mutations in the ethanol inducible ion channel *SL0-1*, the voltage- and calcium-sensitive *BK* channel.	-Suggest a model in which *TAG* levels are important for the development of *AFT* through alterations of the action of ethanol on membrane proteins. -Used a genetic screen for mutations that result in defects in the development of AFT to identify two transcriptional co-repressors, *ctbp- 1* and *pag- 3*, that regulate the ability of animals to develop AFT	No further suggestion was stated by authors	[70]
24	Choi *et al.*, (2016)	Ethanol	Genetic mechanism Membrane permeability	Dose of treatment: 200- ml ethanol solutions; (7% v/v) of ethanol Sample size: 20 worms for each group Duration: 0-10 mins	-Acute exposure to a high concentration (7% v/v) of ethanol changes membrane permeability, as measured by propidium iodide staining, and causes paralysis -Identifie *dptr- 6* as a gene that confers ethanol resistance when mutated -Inhibition of two PTR -encoding genes, *ptr- 15* and *ptr- 23*, andmboa-1, encoding an Acyl Co-A: cholesterol acyltransferase homolog, restored ethanol sensitivity of theptr-6mutant	*ptr -6* mutant may provide a cost- and time-worthy model to screen for new therapeutic treatment of such diseases. - Utilized a targeted candidate approach for the new players and identified two more ptr genes and *mboa-1* as genes involved in membrane integrity and permeability	- Propose the following model for the roles of *PTR* proteins in membrane integrity: *MBOAl/ACAT* synthesizes cholesteryl ester from cholesterol in the hypodermis and PTR- 6 acts to regulate the storage of cholesteryl esters in the hypodermis	[71]

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings	Strength	Limitations/ Suggestion	Ref
25	Zhao et al., (2012)	Ethanol	Genetic mechanism	-Dose of treatment: # -Sample size: # -Duration:#	-Cross-species evidence-based approach is useful to identify candidate genes contributing to alcoholism. -One weighting score matrix could increase FDR based q values for a list of 47genes with a score greater than 2. -These genes were primarily involved in brain responses to ethanol and neural adaptations occurring with alcoholism	-Applied a unique cross-species, evidence-based gene prioritization strategy for genes involved in alcoholism -Test across humans, mice, rosophila and *C. elegans* which is useful for cross-species gene prioritization.	Need more works for further experimental validation in three animal models	[72]
26	Davies et al., (2004)	Ethanol	Genetic mechanism	-Dose of treatment: 0- 500 mM of ethanol -Sample size: 300-600 worms for the experiment -Duration: 10 - 50 mins of exposure	-Explain conserved function of NPY-related pathways in ethanol responses across diverse species. -Allelic variation that alters the functional level of NPR-1, a neuropeptide Y (NPY) receptor like protein, can account for natural variation in the acute response to ethanol in wild strains of *C. elegans.*	- Identified a genetic basis for variation in acute responses to ethanol that occurs in wild strains isolated from distinct geographical regions	Further study need to determine whether differences in the endogenous level of NPY signaling also contribute to the natural variation in behavioral responses to ethanol that are observed in human population	[73]
27	Davies et al. (2015)	Ethanol	Genetic Mechanism	-Dose of treatment: 50 mM of ethanol Sample size: # Duration: #	-Generated transgenic worms that express mutated SL0-1 channels predicted to have the mutated SL0-1 channels predicted to have the insensitive to calcium. -Mutating these domains inhibited basal function of SL0-1 in vivo as neck and body curvature of these mutants mimicked that of the BK null mutant.-Mutating these domains singly or together in SL0-1 had no effect on intoxication in *C. elegans.* -Ethanol activated the SL0-1 channel in vitro with or without these domains.	-Found that ethanol activated the SL0-1 channel in vitro with or without these domains. -Strongly support the idea that combined RCKl and Ca2+ bowl mutation in the human and worm BK channel differentially regulate ethanol sensitivity of channel gating	Further study need to explore other RCKl domain residue differences between the worm and mammalian channel	[74]
28	Cremona et al. (2008)	Ethanol	Locomotion Chemotaxis	Dose of treatment: 0-200-400 mM of ethanol Sample size: 5 worms for each test Duration: 45 mins	Present an automated microsystem for quantitative population experiments.	The use of this system help little post-processing is necessary and multiple worms can be tracked and counted allowing for quantitative standardized population assays over time.	Support for further integration of Support for further integration of information in a high throughput manner for behavioral screens of chemicals and small molecules	[75]

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings	Strength	Limitations/ Suggestion	Ref
29	Johnson *et al.* (2003)	Alcohol	Genetic mechanism	-Dose of treatment: 21 mM 400 mM of ethanol -Sample size: 20-25 worms for each test Duration: 0-500 mins	-Expressing the orthologous *E466K* mutation (*unc-18E465K*) enhanced alcohol sensitivity. (unc-18E465K) enhanced alcohol sensitivity surprisingly independent of *rab-3*. *-unc-18 R39C*, which decreases syntaxin binding, enhanced sensitivity to alcohol in a manner requiring *rab-3*. -Overexpression of *R39C* could suppress partially the reduction in neurotransmitter release in *rab-3* mutant worms, whereas wild- type or *E465K* mutants showed no rescue	Emphasises simple modulation of synaptic strength is unrelated to sensitivity to ethanol and that the functional actions of alcohol are a complex cellular mechanism involving a large spectrum of neuronal proteins	No suggestion stated by authors	[76]
30	Jee *et al.* (2013)	Ethanol	Genetic mechanism Locomotion Behavioural arousal, and tremor Development	-Dose of treatment: 300-400 mM of ethanol -Sample size: 10 worms for each test Duration: 30 mins of exposure and 100 mins of recording	-Isolated a gain-of-function allele of seb-3, a CRF receptor-like GPCR in *C. elegans*, providing an in vivo model of a constitutively activated stress system. - *SEB-3* positively regulates a stress response that leads to an enhanced active state of locomotion, behavioural arousal, and tremor. -*SEB-3* contributed to acute tolerance to ethanol and to the development of tremor during ethanol withdrawal	-Demonstrate functional conservation of the CRF system in responses to stress and to ethanol in vertebrates and invertebrates	No further suggestion given by the authors	[77]
31	Johnson *et al.* (2016)	Alcohol and central nicotine	Genetic mechanism	Dose of treatment: 0-500 mM of ethanol Sample size: 10 ml of worm; 30 worms for thermotolerance test Duration: 10 and 30mins	-Heat shock transcription factor, *HSF-1*, altered sensitivity to both alcohol and nicotine. -These effects were contingent upon the constitutive neuronal expression of *HSP-16.48*, a small heat shock protein (*HSP*) homolog of human a crystalline -Demonstrate the function of *HSP-16.48* in drug sensitivity surprisingly was independent of chaperone activity during the heat shock stress response. -Identified a distinct domain within the N- terminal region of the *HSP16.48* protein that specified its function in comparison to related small HSPs	Establish and characterize a novel genetic determinant underlying sensitivity to diverse addictive substances . - Examined ethanol and nicotine in the test.	More works needed to implicate HSF-1 and small HSPs as a intracellular hub linking genetic predisposition to multiple, complex neurological disorders, including susceptibility to addiction in order to facilitate new avenues for pharmacological intervention to addiction in general.	[78]

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings	Strength	Limitations/ Suggestion	Ref
32	Mathies *et al.* (2015)	Alcohol	Behaviour Genetic mechanism	Dose of treatment: 0-400 mM of ethanol -Sample size: 10 worms/each test Duration: 10 -30 mins of exposure	- Identify a role for the switching defective/sucrose non-fermenting (SWI/SNF) chromatin-remodelling complex in regulating the behavioural response to alcohol in the nematode. -*SWI/SN F* genes are associated with a diagnosis of alcohol disorder (AD) in a human.	Implicate the chromatin remodeling associated with SWI/SNF complex members in the behavioral responses to alcohol across phyla	Suggested an appropriate unit of analysis for association with complex behavioral disorders may be a biological complex rather than an individual gene	[79]

Table 1: Systematic review of original researches using *C. elegans* as a host model organism to study drug-induced responses to effects of stimulants drug (n=39)

Nicotine Substance Study

Nicotine is highly addictive stimulant and widely used globally due to its relaxing and stimulating effects. There are various forms of nicotine in the market including chewing tobacco, cigars, cigarettes and some smoking cessation aids such as electronic cigarettes, nicotine gum and nicotine patches. No less than 27 diverse nicotinic acetylcholine receptor (*nAChR*) sub-units are expressed in the nematode [114,115]. Like in vertebrate, the acetylcholine of the nematode which is vital in essential behavior such as egg-laying, feeding, movement, muscle contraction. Many *nAChR* genes have been identified in the worm [114]. The exposure of nicotine to the worm population induces egg-laying and muscular hyper-contraction. When the worms were continuously exposed into nicotine, egg-laying control ability of the worm is affected which is encoded by *UNC-29* gene [102]. A work conducted by Feng et al. in 2010 demonstrated that the responses of *C. elegans* to nicotine substance is well-ordered by transient receptor potential (*TRP*) proteins by means of *TRP-1* as well as *TRP-2*. Their channel normalize the adversative responses to the examined substance in the experiment. Those analogous functions of nicotinic systems in *C. elegans* as well as the nicotinic-induce behaviors and responses are also similar to the effects of nicotine into human. The *dop-1* or *dop-2* dopamine receptors, have genes encode and cause the reduction of approach to nicotine of the mutant worm strains. The line of attack insufficiency in the acr-15 mutant could be liberated not in muscle but by re-expression in neurons [30]. The findings from the included studies (Table 2) indicated that this host model organism can be used to study rewarding and motivational characteristics of nicotine as well as the basic physiologic effects of the substance. Hence, this can be useful for identifying the molecular underpinning of nicotine addiction and developing new smoking cessation pharmacotherapies for human. Things to be considered to get expected result when using this model include environmental stimuli and drug-associated cues, duration of exposure schedule of dose and route of administration.

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings
1	Feng *et al.*, (2010)	Nicotine	Genetic mechanism and locomotion	- Three dose of treatments: 500 nM, 1.5 µM, 5 µM, - Sample size: 10 worm/sample	-Nicotinic acetylcholine receptor (*nAChR*) family genes mediate nicotine dependence. -Role of TRPC channels in regulating nicotine dependent behaviour. They are important for nicotine-induced calcium responses in commandinterneurons.
2	Musselman *et al.*	Cocaine and methamph	-Behaviour	-- Dose of cocaine: 50 µM; dose of MAP: 50 µM and 500 µM -Sample size: N = 8+ for each cell --Duration :#	-Pairing a distinctive salt cue with a drug (cocaine or methamphetamine) results in a concentration-dependent change in preference for the cue that was paired with the drug during conditioning -*C. elegans* display a conditioned *preference* for environments containing cues previou sly associated with drugs of abuse , and this response is dependent on dopamine neurotransmission
3	T. Matsuura and T. Urushihata (2015)	Nicotine	Genetic mechanism, preference and gustatory plasticity	-Dose of treatment: 0.01-lmM of nicotine -Sample size: 30 worms/spot -Duration: 90 mins	-Chronic nicotine exposure augments gustatory plasticity. -Role of dopamine in the augmentation of gustatory plasticity due to chronic nicotine exposure. -Augment of gustatory plasticity was observed in *tph*- 1 mutants but not in *bas*-1 and *cat*-2 mutants

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings
4	Ward *et al.*, (2009)	Cocaine	-Genetic mechanism -Locomotion Egg-laying	-Dose of treatment: 0.5- 2mM of cocaine -Sample size: 1Oworms -Duration: 1Omins	-Acute cocaine treatment evokes changes in *C. elegans* locomotor activity. -The neurotransmitter serotonin, rather than dopamine, is required for cocaine response in *C. elegans* -The behavioural response to cocaine is primarily mediated by the ionotropic serotonin receptor *MOD-1*
5	Sobkowiak *etal.* (2011)	Nicotine	Behaviour Movement Locomotion	-Dose of treatment: 0.001, 0.01, 0.1, 1, 10 and 30 mM of nicotine -Sample size: A total of 20558 individual worm track s were analyzed -Duration: 0 up to 300mins	-A low concentration (0.001 mM) of nicotine causes a reduction of the speed of movement -Moderate concentrations (0.01 and 0.1 mM) induced acceleration of the mean speed of locomotion of *C. elegans* -High doses of nicotine (above 1 mM) induced slowing down of the movements and, finally, paralysis -Time-dependent analysis revealed that the stimulation phase lasted up to 70 min
6	Taki and Zhang (2013)	Nicotine	Genetic mechanism	-Dose of treatment: OμM (control), 20μM and 20mM nicotine -Sample size: # -Duration: #	-Evaluate the stability of 16 reference gene candidates in *C. elegans* exposed to nicotine *-TBA-1 and CDC-42* were the two most stable reference genes for performing reliable gene expression normalization in the multigenerational impact of nicotine exposure
7	Polli *et al.* (2015)	Nicotine	Locomotion behaviour Genetic mechanism	-Dose of treatment: 6.17 μM and 61.7 μM of nicotine -Sample size: 423 nicotine-free worms and 190 nicotine-dependent worms -Duration: 24hrs	-The linkage between nicotine-induced locomotion behaviour and the regulation of nicotinic acetylcholine receptors -Eleven genes (*lev-1, acr-6, acr-7, acr-11, lev- 8, acr-14, acr-16, acr-20, acr-21, ric-3, and unc-29*) were significantly up-regulated in which worms showed significantly increased locomotion behaviour
8	Katner *et al.* (2016)	Methamphetamine (MAP)	Behavioural and neurochemical changes	-Dose of treatment: 17, 50, and 500 μM -Sample size: 200 μl of worms -Duration: 3Omins	-Determine the long-term behavioural and neurochemical effects of embryonic exposure to MAP in *C. elegans* -Embryonic MAP exposure reduced DA levels in adult *C. elegans* -Food conditioning data suggest that MAP exposed animals can form associations between cu es and food
9	Bonnett *et al.* (2014)	Nicotine	Locomotion Genetic mechanism	-Dose of treatment: 40 μM to 4 mM -Sample size: 20 -40 worms -Duration: 3mins, 20 mins and 24hrs	- Akinesia and freezing are state-dependent and reversible in NALCN-deficient mutants (nca-1; nca-2, unc-79andunc 80) when additional cation channels substitute for this protein. - The NALCN may play an unrecognized role in human movement disorders characterized by akinesia and freezing gait -Nicotine mimics food deprivation and improves movement
10	Robert S. and Andrzej L. (2009)	Nicotine	Genetic mechanism (Genotoxicity)	-Dose of treatment: 0, 1, 10, and 100 μM (-) nicotine -Sample size: # -Duration: 1 hour	-To assess the genotoxicity of nicotine -Nicotine treatment had dose-dependent effects on the level of DNA damage -A high dose of nicotine (100 μM) is genotoxic, while a reasonably low concentration has a protective effect.
11	Gottschalk *etal.*,	Nicotine	Genetic mechanism	-Dose of treatment: 0- 0.2, 1.5, 26.4 and 31 mM nicotine -Sample size: 30 worms	-Performed tandem affinity purification of the levamisole sensitive *nAChR* from *Caenorhabditis elegans*, mass spectrometry of associated components, and RNAi-based screening for effects on in vivo nicotine sensitivity *-TAX-6* function as a negative regulator of *nAChR* activity-five proteins -Positive regulators of *nAChR* activity. -Copine NRA-1 co-localized with the levamisole receptor at neuronal and muscle plasma membranes, and, when mutated, caused reduced synaptic nAChR expression -Loss of *SOC-1*, which acts in receptor tyrosine kinase (RTK) signalling, also reduced synaptic levamisole receptor levels, as did mutations in the fibroblast growth factor receptor *EGL-15*, and another *RTK, CAM-1*.

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings
12	Green *et al.,* (2012)	Nicotine in tobacco	Genetic mechanism	-Dose of treatment: 0-15 ng of nicotine -Sample size: 300 worms/each exposure -Duration: 24hrs	-Identify *6 CS-down-regulated genes* in the innate immune response to PA, RNA interference (RNAi) which are homolog to human genes
13	Ward *et al.,* (2009)	Cocaine	Reproduction (Egg-laying and egg production) Stimulus response Genetic mechanism (gene expression)	-Dose of treatment: 6.17-194.5 mM -Sample size:""600 worms/dose -Duration: 24- 72hrs	-Nicotine significantly affects the organism's response to touch *stimulus* -Chronic nicotine exposure promotes early *egg laying* events and slightly increased egg produ ctions during the first 72h of adulthood -The expressions of 10 (*egl-10, egl-44, hlh-14, ric-3, unc-103, unc-50, unc-68, sod-1, oxi-1, and old-1*) out of 18 selected genes were affected significantly the organism's response to tou ch stimulu s
14	Matsuura *etal.,* (2015)	Nicotine	Gustatory plasticity Genetic mechanism	-Dose of treatment: 0.1, 0.3, 1.0, 3.0, and 5.0 mM nicotine -Sample size: 30 worms/each plate -Duration: 90mins	-Gustatory plasticity is inhibited by acute nicotine exposure. -Nicotine affects the *nAChR* neurons of salt chemotaxis learning and inhibits gustatory plasticity. -Nicotine on locomotory activity, sensitivity, and gustatory plasticity
15	Wescort *et al* (2016)	Nicotine	Locomotion Genetic mechanism	-Dose of treatment: 10, 50, 100, 500, and lOOO µM of nicotine -Sample size: 20 worms/each group ration: 24-48hrs	-Test locomotion speed of worm in acute dose of nicotine exposure: stimulate wildtype but reduce in mutant types *daf-16* -Insulin signalling genes, *daf-2, age-1,pd k-1, akt-1,* and *akt-2* modulate behavioural responses to nicotine in *C. elegans*
16	Waggoner *etal.,* (2000)	Nicotine	Egg-laying Genetic mechanism	-Dose of treatment: 0- 50 mM of nicotine -Sample size: # -Duration: 0-36 hrs	-Prolonged nicotine treatment results in a long lasting decrease in the abundance of nicotinic receptors that control egg-laying. - Nicotinic receptors containing *UNC-29* stimulate egg-laying in *C. elegans* - PKC dependent signalling pathways may promote nicotine adaptation via regulation of nicotinic receptor synthesis or degradation - tpa-1 gene is nicotine-induced down-regulation of *UNC-29* abundance
17	Carvelli *et al.* (2010)	Amphetamine (AMPH)	Genetic mechanism Molecular mechanism	-Dose of treatment: 100 uM of amphetamine -Sample size: at least 54 worms/each test -Duration: 4 mins of exposure	- AMPH produces swimming-induced paralysis in a time- and dose-dependent manner in wild-type (wt) animals but has a reduced ability to generate SWIP in DAT knock out worms (dat-1) - DA efflux through *C. elegans* DAT is required -DA roles: affects learning by for AMPH-induced behaviors and does not mediating the state dependent require DOP-1 signaling. -DA efflux is critical to su staining SWIP behavior by signaling through DOP-3, DOP-4, and DOP-2
18	Sobkowiak *et al.,* (2017)	Nicotine	Genetic mechanism	-Dose of treatment: 0.01 and 1 mM of nicotine -Sample size: # -Duration: ,_,60 mins of nicotine exposure	-Identified dozens of *C. elegans* pro teins that are present exclusively or in higher abundance in either nicotine treated or untreated worms. -Key protein components of nicotine-induced protein complexes and speculate how the different protein modules relate to their distinct physiological roles. nicotine in the course of Alzheimer's disease treatment and contributed to the emergence of Alzheimer's -related peptidase and prion-like proteins in the complex. and proteomic mass spectrometry (MS) analysis procedure should also be applied to the investigation of the multi-protein complexes. -Mechanisms of nicotinic acetylcholine receptor -Provide solid foundation for (nAChR) signalling and further exploration of the nicotine trafficking in cells

No	Author(s) Year of published	Substance Stu dy	Research focus	Concentration used and sample size traits Duration of study	Principle findings
19	Taki *et al.,* (2013)	Nicotine	Genetic mechanism Locomotion, speed, body bends	-Dose of treatment: 20 µm of nicotine -Sample size: # -Duration: 31 hrs of nicotine exposure	-It is the first study to reveal that *nicotine addiction is heritable.* -Nicotine was associated with changes in sinu soidal *locomotion, speed,* and *body bends* in L4 larvae in all three tested generations FO, Fl, F2
20	Sellings *et al* (2013)	Nicotine	Genetic mechanism Development	-Dose of treatment: 1 um- 0.5M of nicotine -Sample size: at least 4 worms/test -Duration: 120 mins	-Nicotine acts as a rewarding substance in *C.elegans.* -The nicotinic receptor antagonist mecamylamine, the smoking cessation pharmacotherapy varenicline, mutation of the *dop-1* and *dop-2* dopamine receptors, and mutations of either *acr-5* or *acr-15*, two nicotinic receptor subunit genes with sequ ence homology to the mammalian a7 subunit, all reduced the nicotine approach behaviour. -The approach deficit in *acr 15* mutants was rescued by selective re-expression in a subset of neurons, but not in muscle
21	Rose *et al.* (2013)	Nicotine	Locomotion Genetic mechanism Development	-Dose of treatment: 30 µM of nicotine -Sample size: # -Duration: ,...,10 mins/test period	-Use RT-PCR to test expression of acr-16 (a nicotinic receptor subunit) and a *P-like GABA A* receptor subunit, *gab-1.* -Spontaneous motor behaviour and receptor expression are differentially modulated by nicotine exposure during larval development and/or zygote formation. -Results indicate that whether the nicotine exposure condition resulted in an up- or down regulation of *acr-16* or *gab-1* expression, the mechanism by which *gab-1* expression appears to be consistently elevated above *acr-16* expression persists across conditions.
22	Jayanthi *et al.,* (1998)	Cocaine & Ampheta mine	Genetic mechanism and pharmacolgy	-Dose of treatment: 0-60nm -Sample size: # -Duration: #	-*C. elegans* utilizes the catecholamine dopamine (DA) as a neurotransmitter to control or modulate movement and egg-laying. -T23G5.5 locus as encoding a functional catecholamine transporter responsible for DA inactivation in vivo.
23	Towers *et al.,* (2005)	Nicotine	Egg-laying behaviour Pharyngeal pumping Genetic mechanism	-Dose of treatment: # -Sample size: # -Duration: 1 hour of exposure	-*lev-8* encodes a novel nicotinic acetylcholine receptor (*nAChR*) subunit (previously designated A CR-13), which has functional roles in body wall and uterine muscles as part of a levamisole-sensitive receptor. -*LE V-8* is a levamisole receptor subunit and exhibits the most diverse expression pattern of any invertebrate nAChR subunit studied to date.
24	Francis *et al.,* (2005)	Nicotine	Genetic mechanism	-Dose of treatment: # -Sample size: # -Duration: #	-Identify two genes required for the major excitatory current found at the worm neuromuscular junction (NMJ): -*acr-16*, which encodes a nicotinic AChR subunit homologous to the vertebrate *a7* subunit -*cam-1,* which encodes a *Ror* receptor tyrosine kinase

Table 2: Systematic review of original researches using *C. elegans* as a host model organism to study drug-induced responses to effects of depressants drug (n=24)

Ethanol Substance Study

As stated by World Health Organization (WHO), in addition to health benefits from drinking alcohol with slight drinking, there are a number of severe diseases in common with other social problems caused by alcohol use globally. It is said to cause about 6% of all deaths of the community worldwide [118]. The users are very easy to be addicted or alcohol dependence. Remarkably, detail of the mechanism of genes initiative ethanol misuse as well as syndromes related to alcohol use is still not fully understood. Recently, *C. elegans* have been used in the experiments to investigate alcohol-induced responses of the worm after exposed to ethanol (EtOH) including physical behavior, locomotion, egg-laying, body bend amplitude [116]. When the concentration of alcohol in the internal tissue of *C. elegans* reaches level that same with that one related to intoxicating blood alcohol volume in individuals, it will cause the depressant effect on the locomotion of the worm [65]. Thus far, the orthologs of identified genes that stimulate behaviors against alcohol use in the nematode have been concerned in AUD (alcohol use disorders) in humans [117]. Like in vertebrate, the dopamine system play crucial role in EtOH-induced behavioral effects in the worm body. In the mutant strain of the worm, with the tyrosine hydroxylase or vesicular monoamine transporter (*cat-2 or cat-1, respectively*), state dependent learning is induced by EtOH exposed. In 2009, Lee and the co-workers design experimental paradigms to test EtOH preference mediated through the serotonin and dopamine systems [66]. From that, these behavioral paradigms were used by many researchers later on to categorize mutations in targeted genes that influent behavior reactions to ethanol in the worm. Then, preserved homologous genes might be further investigated regarding the effects of EtOH-related behaviors in other higher organisms and human to a better understanding and treatment for alcohol use disorder.

Davies *et al.* (2003) indicated that mutations in the gene *SLO-1*, a highly conserved gene coding BK potassium channel that is homologous in human one, produced resistance to the locomotion effects of EtOH [48]. The BK potassium channel sub-serves behavioral response not only in this worm species, but also in other species including human [31-33-48]. Finding from a research conducted by Davis and his co-workers in 2014 identified that specific residue (*T381I*) on that channel confers selective and dramatic resistance to the behavioral effects of examined substance [59]. Consequently, this channel can serve as a target to be used to identify and develop new treatment for alcohol abusers. After long-term repeated or chronic exposed to EtOH with the same concentration, the worms reach tolerance stage [33]. Hawkins *et al.* (2015) found that EtOH-induced muscle hyper-contraction in mutant strains (*CHA-1* and *UNC-17*) is dependent on cholinergic signaling [51]. In the Na+/K+ ATPase mutant strains (*EAT-6*), tolerance symptom was not found as it was evident in wild-type strains [83]. Also reported by Raabe *et al.* in 2014, long-chain polyunsaturated fatty acid affects cholinergic functioning and mutant deficient in this kind of fatty acid display shortfalls in the increase of acute functional tolerance together with the initial sensitivity to EtOH [83]. From that, through orthologous mechanisms, the data from fatty acid metabolism, cholinergic systems and tolerance effects of EtOH in the worm can be considered for further investigation in higher organism and in human.

The mechanism of *BK* channel trafficking is a vitally supervisory mechanism for neural function and synaptic transmission. An endoplasmic reticulum (ER) membrane protein called *ERG-28* control the trafficking of *SLO-1* BK channels to the plasma membrane from the *ER* [63]. This research fining also indicated that *ERG-28/DDI-1* regulates overall *SLO-1* levels contingent on physiological necessity or cellular stress. The researchers also suggested that more studies to assess the functional conservation between mammalian and the nematode *ERG28* proteins are essentially needed [63]. Recently, Adskin *et al.* in 2017 concluded that knockdown of a *COL6A3* ortholog in the worm moderates ethanol sensitive properties. In their experiment result, they identified substantial relationship in *COL6A3* and redolent relationship in two heretofore involved loci, *RYR3* and *KLF12*. They also indicate the role of genes *LOC339975, RYR3, KLF12* and *COL6A3* in response to EtOH across multiple species and/or alcohol user risk in human. However, it is poorly understood about functions of long noncoding RNAs [69]. Generally, *C. elegans* model organism can be effectively used for identification of epigenetic factors as well as alcohol-related regulation genes and/or proteins that might function as prospect targets to alcohol use disorder treatment and medication (Table 3 for systematic review)

Categories	N	%
Publication year		
1998	1	1.59
2000	1	1.59
2003	2	3.17
2004	3	4.76
2005	4	6.35
2007	2	3.17
2008	4	6.35
2009	5	7.94
2010	3	4.76
2011	3	4.76
2012	6	9.52
2013	9	14.29
2014	4	6.35
2015	9	14.29
2016	4	6.35
2017	3	4.76
Theory		
Discussed	63	100
Not discussed	0	0
Substance study		
Ethanol	39	61.90
Nicotine	19	30.16
Cocaine	2	3.17
Amphetamine	1	1.59
Methamphetamine	1	1.59
Cocaine and amphetamine	1	1.59
Study focus		
Genetic mechanism	56	88.89
Locomotion	18	28.57
Behaviour	12	19.05
Mobility	2	3.17
Fertility	3	4.76
Preference	3	4.76
Development	8	12.70
Reproduction –egg laying	9	14.29
Life expectancy	1	1.59

Categories	N	%
Follow up length of the study		
Acute (mins of exposure)	49	77.78
Chronic (hours of exposure)	14	22.22
Dosage of treatment		
Ethanol		
Unit of % v/v ethanol		
0-7% v/v ethanol	3	7.69
0-20% v/v ethanol	2	5.13
Unit of mM		
0-100mM	3	7.69
0-400mM	22	56.41
0-500mM	6	15.38
0-2M	3	7.69
Stimulants		
Unit of mM		
0-500nM	2	8.33
0-500µM	8	33.33
0-50mM	14	58.33

Table 3: Characteristics of papers included in the systematic review

Cocaine Substance Study

Although cocaine has been clinically used as anesthetic in ophthalmology, it is not ordinarily prescribed as a stimulant with its property, therapeutically. Cocaine was initially used with recreation purpose, however, it is more addictive than amphetamine. Like in vertebrates, cocaine affects to dopamine transporter (*DAT-1*) of the *C. elegans* and the dopamine neurotoxin 6-hydroxydopamine of this worm triggers dopamine neuronal degeneration [113]. The concentration of 50 -60 mM of cocaine in the experiments showed a reduction in locomotion and remarkably this effect mediates through serotonin system, not in dopamine system [88,90,108]. A study conducted by Musselman and his co-workers (2012) designed a Pavlovian chemosensory cue-conditioning paradigm to investigate reward-like behaviors of cocaine (and also of methamphetamine) in the nematode [42]. Cocaine affects preference for either a food cue or salt. The preference effect is not expressed in both *cat-1* and *cat-2* dopamine - scarce mutants. Generally, all the studies suggested that *C. elegans* can be effectively used to examine the rewarding properties and behavioral responses of cocaine.

Amphetamine and Methamphetamine Substance Study

Amphetamine produces its clinical effect by modulating central and peripheral catecholamine neurotransmitter system consequently causes sympathomimetic effects. Amphetamine and methamphetamine can be eliminated via real and hepatic clearance with estimated half-life ranging from 06 to 12 hours. A study was conducted by Carvelli *et al.* (2010) indicated that dopamine efflux through the nematode DAT is requisite for amphetamine – prompted behaviors and not need *DOP-1* signaling [103]. Similar to cocaine, methamphetamine has addictive properties by affecting on dopamine system [111]. It inhibits uptake and induces release as well as biogenic amine neurotoxicity [112]. The exposure concentration of methamphetamine (ranging from 0-50-500 mM of methamphetamine) into *C. elegans* with the duration of 30 minutes and 01 hour shown effects on their behaviour, egg-laying, genetic mechanism and preference [42,94]. The concentration of 50-500mM of methamphetamine trigger increase in preference of the worm for either food cue or a salt. Like cocaine, the preference effect is not expressed in both cat-1 and *cat-2* dopamine-deficient mutants [42]. In 2012, Musselman and his co-workers designed a Pavlovian chemosensory cue-conditioning paradigm to investigate reward - like behaviors of methamphetamine in the nematode. In the existence of dopamine, that response of methamphetamine was liberated in those mutants [42]. The relationship between the appetitive/addictive and neurotoxic properties of methamphetamine was not fully investigated [94]. *C. elegans* is a respectable model platform used to investigate this relationship in order to diminish the neurotoxic result of this substance. The result from the studies indicated the role of dopamine system in mediating methamphetamine rewarding effects, and both neurotoxic and rewarding effects of this substance can be identified in *C. elegans* model organism.

Conclusion and Future Recommendation

Basically, effective pharmacotherapies for addiction's treatment are needed to minimize the consequences of this issue to human health, social and economic aspects. Using systematic review methodology for this review project, summarizing and analyzing from the original research findings, the target/gene underpinning drug-induced behavioral responses among drugs dependence have partially been identified. Since then, the research outcomes, *C. elegans* model rationality and its usefulness for medication progress and expansion can be improved, especially in drug of abuse healthcare issues. The large number of genes identified as well as genetically drug-induced responses have been investigated demonstrates that *C. elegans* – an invertebrate model organism (IMO)– has an analytical power to be used to investigate genetic and molecular mechanisms principal nervous system reactions to drugs. It is indicated as one of the fundamental investigational stands for ascertaining and successively examining new genetic factor that moderate stimulant and depressant-related behaviors. The differences between studies in human and in IMO show

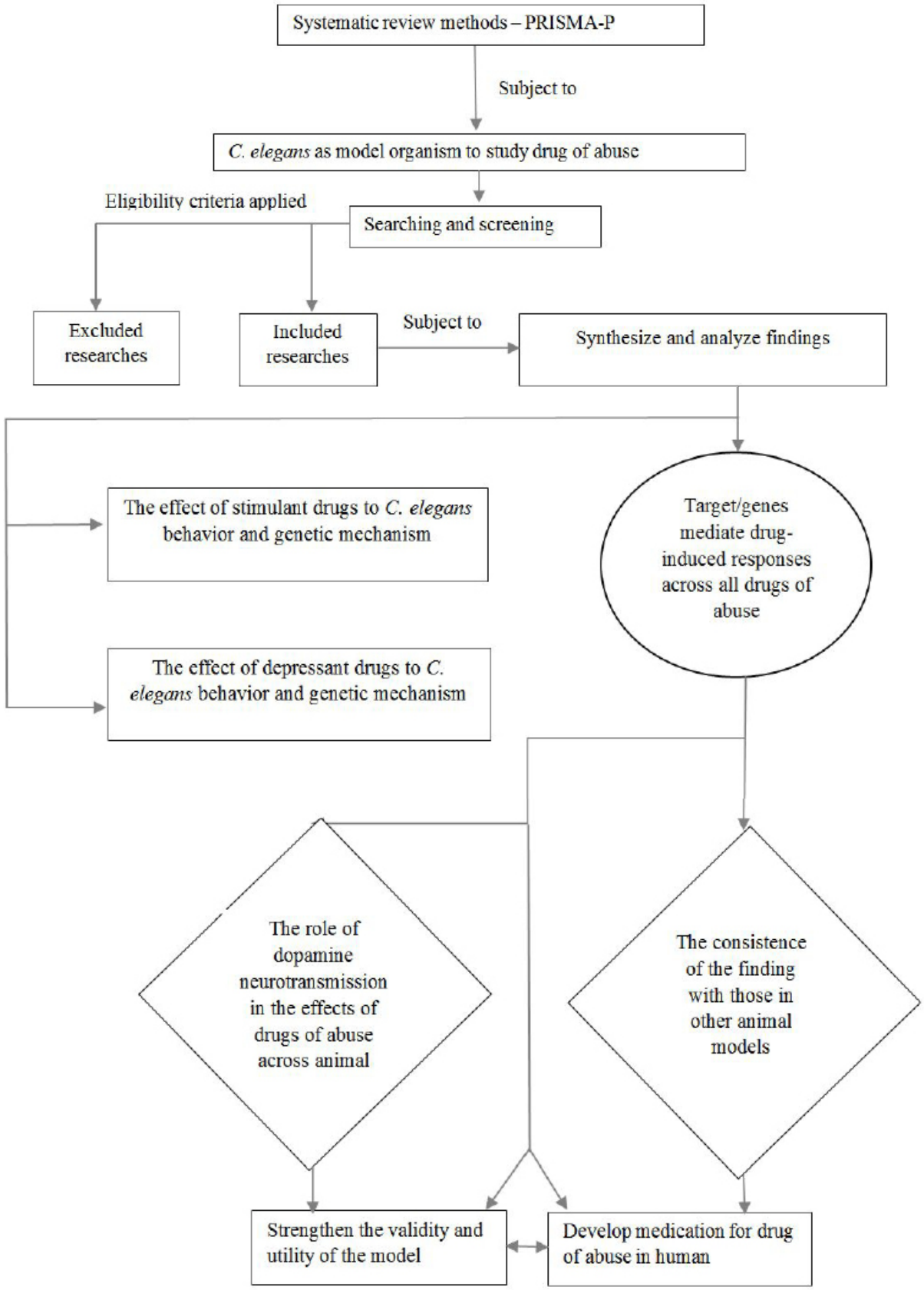

Figure 2: Generally Proposed Research Outcomes of the Systematic Review

that not every drug behavioral-related gene recognized in IMO are similar to human ones. However, orthologs of the identified behavioral-related genes of included papers in this review might be main candidates for focused research for human drug abuse and addiction. Data from researches show that the consistence in finding of dopamine system involvement in several classes of abuse drugs help support a preserved and animated role for dopamine in the possessions of substances of addiction through organisms. From that, further model refinement/ improvement may expand the rationality of the model in research, and other applications of the powerful molecular genetic practices used in the worm may develop its usefulness to support in the proof of identity of new targets and new way of addiction treatments in human (Figure 2). Future recommendation with more future works are needed to contribute to people understanding of molecular/genetic mechanisms that impact behavioral response to EtOH and/ or stimulant substances in higher animal and human kind. Gene and genetic mechanism study in the future need to identify what specific genes influent the developmental against adult physiological processes associated with behavior triggered by alcohol as well as the interaction between the gene-driven developmental and adult physical and biological progressions which are influent by alcohol-related behavior. Moreover, novices and experts should pay attention more on the most important areas of the nervous system in which the genes function and what neurotransmitter systems modulated by the genes. Therefore, together with genetic findings from human regarding alcohol and drug abuse, a fully-fledged entity of understanding about behavioral-genetic studies of those substances plays a crucial role to lead to a much completed perception of drug abuse and addiction, its positive treatments as well as its diagnosis.

Acknowledgement

The author's sincere appreciation goes to Vietnam International Education Development – Ministry of Vietnam Education and Training and Dong Nai Technology University – DNTU for their funding and support during the research by scholarship under the Project 911 (2016-2020) to study drug-induced responses to the effects of stimulant and depressant drugs using *C. elegans* as host model.

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