Journal of Applied Pharmaceutical Science Vol. 12(05), pp 187-195, May, 2022
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2022.120517
ISSN 2231-3354

The effects of ginger (*Zingiber officinale*) rhizome extract on ethanol-induced behaviors in *C. elegans*

Rafael Vincent Mercado Manalo, Bianca Louise C. Lapuz, Krisha K. Lim, Katelyn Edelwina Y. Legaspi, Patricia Marie M. Lota, Francine Melissa B. Lucas, Abdelaziz N. Maldisa, Vinson C. Malipot, Christine Bernadette O. Lo, Allen Khriztofer P. Lichauco, Paul Mark Baco Medina*

Biological Models Laboratory, Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila, Manila, Philippines.

ARTICLE INFO

Received on: 17/09/2021
Accepted on: 11/02/2022
Available Online: 05/05/2022

Key words:
Alcohol, addiction, tolerance, withdrawal, *C. elegans*.

ABSTRACT

Ginger has been used traditionally as a hangover cure in Asia, with potential GABAergic and anti-withdrawal properties. However, studies on its use remain scarce, despite the increasing prevalence of alcohol disorders. Hence, we sought to determine the effects of ginger (*Zingiber officinale*) rhizome extract (GRE) on alcohol-induced behaviors in *Caenorhabditis elegans*. Nematodes were exposed to either vehicle (KPO_4) or ethanol (EtOH) ± GRE at 10-fold dilutions (1, 10, 100, 500, and 1,000 µg/ml) and were tested for acute depression and tolerance, withdrawal, and associative learning. We found that GRE at 500 and 1,000 µg/ml acted as an acute depressant of reversals, omega turns, and sensations to light and nose touches. Interestingly, GRE at the same concentrations also increased the recovery of nematodes at 40 minutes, suggesting an improvement in tolerance. GRE was also able to reduce withdrawal-induced deficits in locomotion and decreased learned preference to EtOH by 53% at 1,000 µg/ml. Altogether, these data show that GRE is a short-acting depressant that improves tolerance, tapers withdrawal-induced behavioral deficits, and disrupts learned preference to EtOH in *C. elegans*.

INTRODUCTION

About 3.3 million deaths globally were attributable to alcohol use and abuse in 2012. In the Philippines, 4.6% of males and females aged 15 and above are diagnosed with alcohol use disorder, and 2.9% are categorized under alcohol dependence. However, no national guidelines, action plans, or national monitoring systems yet exist in the Philippines for alcohol use and abuse. Hence, it is mandatory to invest in research on alcohol to influence and promote therapy and management in this field (World Health Organization, 2014).

Of the acute concerns, *hangovers* and hospital management prove to be the most immediate. *Hangovers* can lead to lower performance, less efficiency at work, and risks for catastrophes such as in driving or navigation (Penning *et al*., 2010). Meanwhile, intoxicated patients admitted to the hospital with comorbid concerns can be challenging to manage. In an article published in the Emerging Medicines Journal, alcohol-related emergency department attendances are at 12%–15% on average and peak on Friday and Saturday evenings, when up to 70% of all attendances can be alcohol-related (Irving *et al*., 2017). Therefore, performing triage while subsequently taking history and providing management can be cumbersome, due to decreased sensorium and motor function.

Fortunately, ginger (*Zingiber officinale*) has been traditionally used to promptly manage the symptoms of *hangovers*, often taken after drinking to reduce nausea and vomiting. In fact, ginger has been shown to be as effective as metoclopramide in preventing nausea and vomiting (Ernst and Pittler, 2000), which may point to its use for *hangovers*. In 2014, ginger was also shown

*Corresponding Author
Paul Mark Baco Medina, Biological Models Laboratory, Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila, Manila, Philippines.
E-mail: pmbmedina @ post.upm.edu.ph

© 2022 Rafael Vincent Mercado Manalo *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/).

to prevent morphine-induced addiction in mice (Torkzadeh-Mahani *et al*., 2014), suggesting that ginger might be capable of managing both the acute and chronic effects of substance use and abuse.

In this study, we aimed to explore these effects of ginger rhizome extract (GRE) on alcohol-induced behaviors and withdrawal symptoms in *Caenorhabditis elegans* (*C. elegans*) and to establish methods to allow its use as a high-throughput screening model for drugs with therapeutic value on alcohol use and abuse, which is supported by previous reports (Davies *et al*., 2003; Lee *et al*., 2009). Here, we show that GRE is a consistently acute depressant at 1,000 µg/ml but promotes tolerance over time compared with EtOH and vehicle alone. Furthermore, GRE restores deficits in locomotion induced by acute withdrawal of EtOH after prolonged exposure and disrupts learned preference of *C. elegans* to EtOH coupled with OP50 as a desirable stimulus.

MATERIALS AND METHODS

All works were carried out in the Biological Models Laboratory of the Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila. The study was conducted at 20°C and under the precautions of Biosafety Level 1, as approved by the biosafety committee of the university.

Collection of plant material

Two kilograms of *Z. officinale* rhizomes was obtained from Legazpi Sunday Market, Makati, which directly sources ginger rhizomes from La Trinidad, Benguet, Philippines. A sample of the rhizome was then sent to the Bureau of Plant Industry for taxonomic classification.

Preparation of ethanolic GRE

Ginger was minced and air-dried for 7 days, prior to soaking in 95% ethanol for another 7 days to minimize dilution of the solvent, in a ratio of approximately 2.05 kg:2 l ethanol (EtOH). The solvent was then decanted and subsequently filtered via double-layered *Whatman* twice and was then sent for rotary evaporation at the Natural Products Laboratory of the Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila. The moist extract was air-dried for 2 days and then heated for 2–3 hours at 55°C, with the dry weight obtained for dissolution in KPO_4 buffer of pH 6.0. In general, seven (7) solutions were prepared: (-) vehicle (1M KPO_4 pH 6.0 only), (+) vehicle (1M KPO_4 + 500 mM EtOH), 1 µg/ml + 500 mM EtOH, 10 µg/ml + 500 mM EtOH, 100 µg/ml + 500 mM EtOH, 500 µg/ml + 500 mM EtOH, and 1,000 µg/ml + 500 mM EtOH. Phytochemical analysis of the extract was not carried out.

Preparation of nematode growth media (NGM)

Nematodes were maintained in NGM plates prepared at the Biological Models Laboratory of UP Manila. Briefly, 3 g NaCl, 2.5 g peptone, and 20 g agar were dissolved in 1 l of dH_2O and autoclaved at 121°C for 1 hour and then cooled to 60°C prior to the addition of 1 ml each of 1M $MgSO_4$, 1M $CaCl_2$, and 5 mg/ml cholesterol in ethanol. After thorough mixing, the medium was then poured into Petri plates using sterile techniques and stored at 4°C until further use. NGM plates were then seeded with Escherichia coli strain OP50 on the day of assaying.

Age synchronization of *C. elegans* strain N2

Nematodes (N = 20) at stage L4 to young adulthood were transferred into prepared NGM plates seeded with *E. coli* strain OP50. This range in age is considered as a limitation in the morphological characteristics of the nematode at this time, with minimum variation in physiology. Worms were worm-picked until each treatment group had N = 20 per trial. In each assay, each worm acted as a biological replicate.

Lethality assay in *C. elegans*

Toxicity assay was based on the protocols of Williams and Dusenbery (1988) and Katiki *et al*. (2011), with slight modifications (Katiki *et al*., 2011; Williams and Dusenbery, 1988). Briefly, *C. elegans* (N = 20) were exposed separately to 10-fold dilutions in concentration (0.1, 1, 10, 100, 500, and 1,000 ug/ml) or vehicle (1% DMSO) and were followed up after 24 hours to check for acute exposure, to determine if the concentrations chosen did not cause significant attrition and affect statistical power. Percent attrition of 20% or more leads to lower statistical power because of attrition bias (Dumville *et al*., 2006); therefore, concentrations were used for subsequent assays only if the survival rate was >85% after 24 hours. All concentrations were nonlethal in this study.

Working concentrations of ethanol exposure

All *C. elegans* nematodes were exposed to 500 mM ethanol, which corresponds to a nematode internal concentration of ~22 mM (Davies *et al*., 2003). The minimum blood ethanol concentration in humans to induce intoxication is 0.1% or 21.7 mM, which corresponds to the worm concentration. This concentration was therefore used to interpret the results of exposure more reliably.

Acute depression and tolerance

Twenty *C. elegans* worms were exposed to 500 mM ethanol (EtOH) for 20 or 40 minutes, respectively, either with vehicle (KPO_4 buffer pH 6.0) or with GRE. The GRE concentrations used were serially diluted at 10-fold dilutions (1,000, 500, 100, 10, and 1 µg/ml), which were all coadministered with EtOH. Then, five parameters each for locomotion (body bending, short reversals, long reversals, total reversals, and omega turns) and sensation [plate tap, light touch (head), light touch (tail), nose touch, and harsh touch] were recorded per worm per treatment group. All parameters of locomotion were obtained by first transferring each nematode singly to blank NGM and allowing them to roam for 60 seconds to minimize startle behavior (Zhao *et al*., 2003). Then, each worm was individually videotaped for 20 seconds and scored for bending, reversals, and omega turns. For general sensation, plate tap was carried out by tapping the blank NGM plate five consecutive times and recording a reversal, where observed. Should a reversal not be observed, a second set of five consecutive taps were done before recording a negative score. For light touches to the head and tail, a strand of hair fixed to a sturdy handle was used to brush the area closest to the pharynx (head) and anus (tail). Similarly, nose touch was carried out by laying the hair in front of the worm in advance of its direction and recording

its motion after touching the hair with its nose. Lastly, harsh touch was carried out by carefully poking the midbody region of the worm with a fixed nichrome wire, with the response (forward or backward motion) similarly recorded (Fig. 1).

Alcohol withdrawal

To induce withdrawal, the method of Scott *et al.* (2017) was adopted, with slight modifications. Briefly, worms were pretested for locomotion and were then exposed to EtOH ± GRE for 24 hours. Then, each worm was withdrawn to a neutral NGM seeded with OP50 for 1 hour. After that, each worm was transferred individually to blank NGM plates and tested for the five locomotion parameters similarly aforementioned in the depression and tolerance assays.

Induction of ethanol preference

Ethanol dependence in *C. elegans* was induced using the methods of Lee *et al.* (2009), with slight modifications. Briefly, stage L4 to young adult worms were age-synchronized into NGM Petri plates and starved for 12 hours. After 12 hours, all the plates were half-seeded with OP50, and varying concentrations of GRE ethanolic concentrations (1, 10, 100, 500, and 1,000 µg/ml) with 500 mM of ethanol were placed per plate. The control plate contained KPO_4 with 500 mM ethanol. All plates were sealed and taped for at least 6 hours.

Assaying for ethanol preference

Worms induced to prefer ethanol were transferred to plates with four quadrants each according to the protocol of Lee *et al.* (2009). Briefly, two quadrants diagonal to each other (labeled B and C) contained a chemoattractant—10 µl of 0.1 M sodium acetate ($NaCH_3COO$)—while two other quadrants diagonal (A and D) contained 10 µl of 500 mM EtOH. Worms were then placed in the middle of the plate and were allowed to roam the quadrants for 1 hour, after which the proportion of nematodes in the ethanol quadrants was scored. In our preference assay, a preference index (PI) is calculated as [(number of animals in quadrants A and D)—(number of animals in quadrants B and C)] / total tested animals. In all quadrants, 1 µl of a paralytic—1M sodium azide (NaN_3)—was placed near the attractants to immobilize the worms shortly after the quadrant had been entered. Furthermore, plates in the queue for scoring were placed at −40°C to prevent worms outside any quadrant from contributing to the overall PI after the allotted time (1 hour). The PI was then calculated as follows (Fig. 1).

Statistical analyses

For all treatment groups, data were statistically analyzed for normality via the Shapiro–Wilk test, followed by a one-way analysis of variances *post hoc* Bonferroni–Holm test for parametric data and a Kruskal–Wallis *post-hoc* Conover test for nonparametric data, $N = 20$ for each treatment. "*" denotes significance at $p < 0.05$. Data are presented as mean ± SE.

RESULTS

Acute exposure to GRE in *C. elegans*

When *C. elegans* was exposed acutely (20 minutes) to 500 mM ethanol, no significant changes were observed in body bending, short reversals, and omega turn rates—a finding that contrasted previous findings. However, an increase in reversal rate was observed, accounted for almost exclusively by an increase in long reversal frequency (Fig. 2). It has been previously shown that the probability of omega turns increases with reversal duration, so expectedly omega turn rate should increase (Zhao *et al.*, 2003). The lack of increase in omega turn rates suggests (1) a loss of

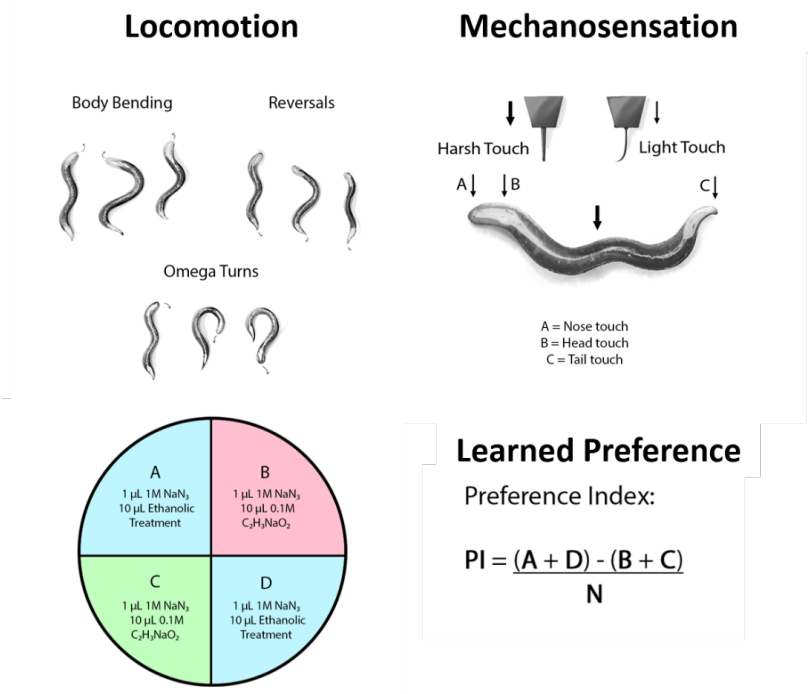

Figure 1. Locomotion and mechanosensation is monitored in *C. elegans* by tracking voluntary movements and responses to mechanical stimuli while preference is determined via chemotaxis.

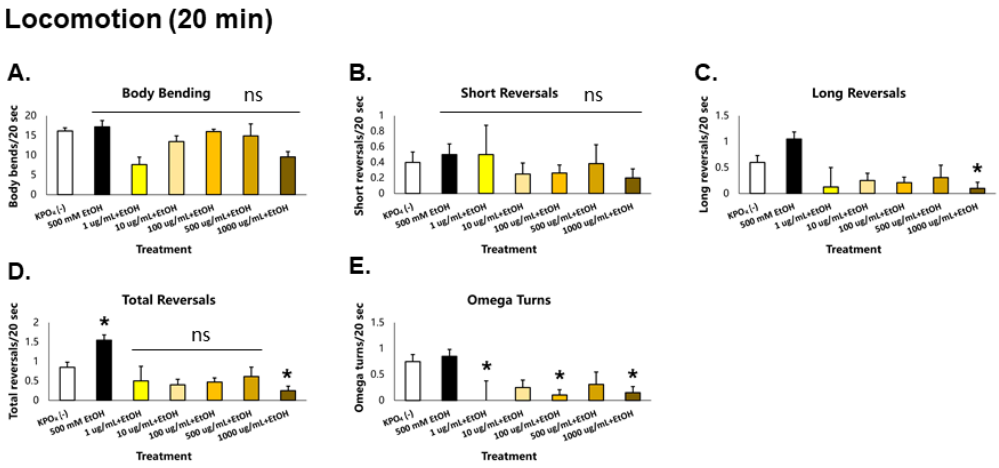

Figure 2. GRE acts as an acute depressant of locomotion in *C. elegans*. Asterisks (*) denote significance at $p < 0.05$ versus vehicle.

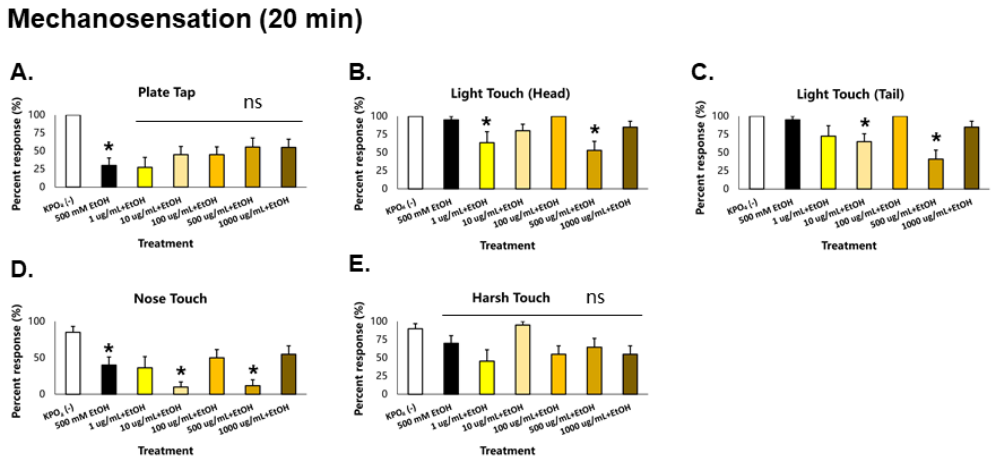

Figure 3. GRE acts as an acute depressant of mechanosensation in *C. elegans*. Asterisks (*) denote significance at $p < 0.05$ versus vehicle.

coordination between long reversals and omega turns and (2) the presence of long reversals without a change in direction, which indicates an increase in meaningless locomotion. The concentration of ethanol used in this study (500 mM) was shown to result in an internal concentration of approximately 0.1% in *C. elegans*; in alcohol intoxication, a blood concentration of 0.1% leads to symptoms indicative of either euphoria or excitement—characterized by a delayed reaction time, loss of coordination, and vision and balance problems, among many (McIntire, 2010; Olson *et al*., 2013). It is interesting to note that these were also observed in the *C. elegans* model upon ethanol exposure, which suggests conservation of behaviors during alcohol intoxication.

When a response was elicited via physical stimulation, worms acutely exposed to ethanol showed a striking 75% reduction in plate tap response, which indicates loss of general sensation (Fig. 3). This suggests depression in the mechanoreceptor neurons ALML, ALMR, AVM, PLML, PLMR, and PVM which mediate general sensation and light touch (Goodman, 2006). More than a 50% reduction in nose touch response was also observed, which suggests depression of the ASH neuron—whose function is monitored via the nose touch response. Minor reduction in harsh touch response was also observed, which suggests depression of the PVD neuron in *C. elegans*, which is a nociceptor neuron in the chosen animal model. These mechanoreceptors deliver signals to AVA, AVD, AVB, and PVC interneurons: AVA and AVD deliver signals to DB and VB motor neurons which causes backward motion; AVB and PVC deliver signals to DA and VA motor neurons which causes forward motion (Goodman, 2006).

In worms exposed to GRE, significant decreases in the magnitude of all parameters of locomotion were observed, especially in the 1,000 ug/ml group, which were also greater than those exposed to ethanol alone. In addition, all parameters of sensation were depressed but were greater in the 500 ug/ml group. These results suggest that GRE is generally a depressant, which seems more potent than the concentration of ethanol used. Likewise, GRE cannot rescue *C. elegans* from acute ethanol-induced aberrations in sensation and locomotion because ethanol is also a depressant at moderate to high doses.

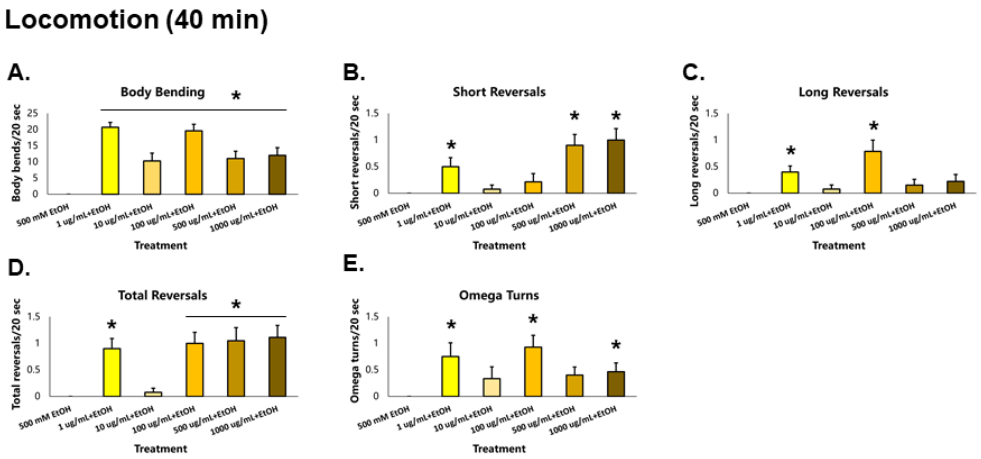

Figure 4. GRE improves the recovery of locomotion in *C. elegans* possibly by improving tolerance. Asterisks (*) denote significance at $p < 0.05$ versus EtOH alone.

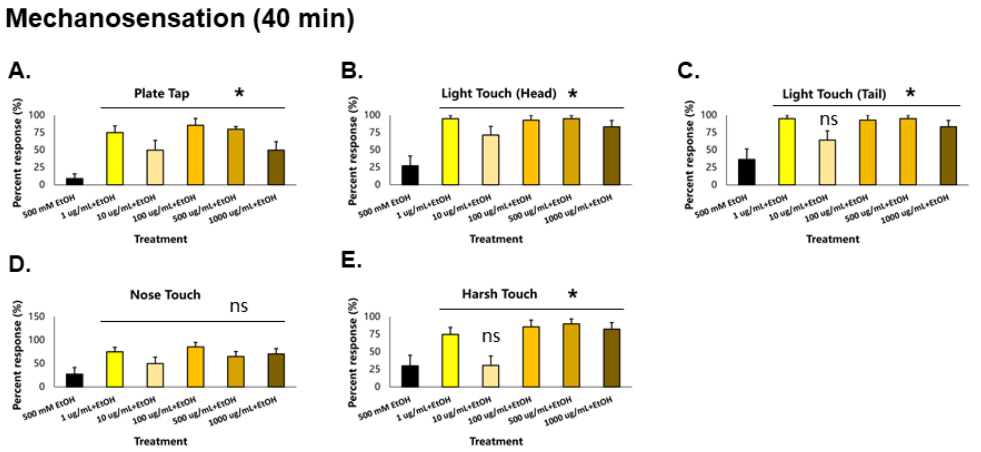

Figure 5. GRE also improves the recovery of mechanosensation in *C. elegans*. Asterisks (*) denote significance at $p < 0.05$ versus EtOH control.

Among all parameters of locomotion, there were no observed movements in the 500 mM ethanol control group. Comparing results from the motor depression assay conducted after 20 minutes of alcohol exposure (Fig. 3A–E), a drastic decrease in locomotion from levels suggestive of reversal duration and omega turn rate coordination loss and meaningless locomotion to levels of complete absence of movement signifies that ethanol tolerance has yet to be induced in the worms. This finding follows that of a previous study which showed that the N2 strain of *C. elegans* develops alcohol tolerance slowly (Davies *et al*., 2004).

Extended exposure to GRE to assess tolerance in *C. elegans*

At 40 minutes, nematodes exposed to GRE were again assayed for both locomotion and sensation versus those exposed to ethanol alone, to determine the effects of GRE on worm tolerance. For body bends, all treatment groups were significantly higher than the ethanol group ($p < 0.000001$) after the Kruskal–Wallis *post-hoc* Conover test. For short reversals, the 1, 500, and 1,000 µg/ml treatment groups were significantly higher than the ethanol group ($p = 0.000218$), while treatments 1 and 100 µg/ml for long reversals were significantly higher ($p = 0.001490$). Total reversals showed significantly higher results across all treatments ($p = 0.000138$) except for 10 µg/ml. Lastly, omega turns were shown to be significantly higher in treatments 1, 100, and 1,000 µg/ml ($p = 0.012692$). Significantly higher locomotion parameters for the tolerance assay were seen most consistently in the 1 µg/ml followed by the 100 and 1,000 µg/ml treatment groups. Other treatment groups not able to generate significant difference with the ethanol control group, especially the 10 µg/ml group, could be attributed to a sampling error, wherein the worms might have been injured in the process of worm-picking, leading to decreased movement, if any. Nevertheless, GRE treatment significantly increased the recovery of locomotion to an otherwise depressed worm with only ethanol as its exposure (Fig. 4).

Similar to locomotion, mechanosensation parameters (plate tap, light touch head, light touch tail, nose touch, and harsh touch) of the control group worsened compared to those taken at 20 minutes (Fig. 5A–E). This signifies that ethanol tolerance has

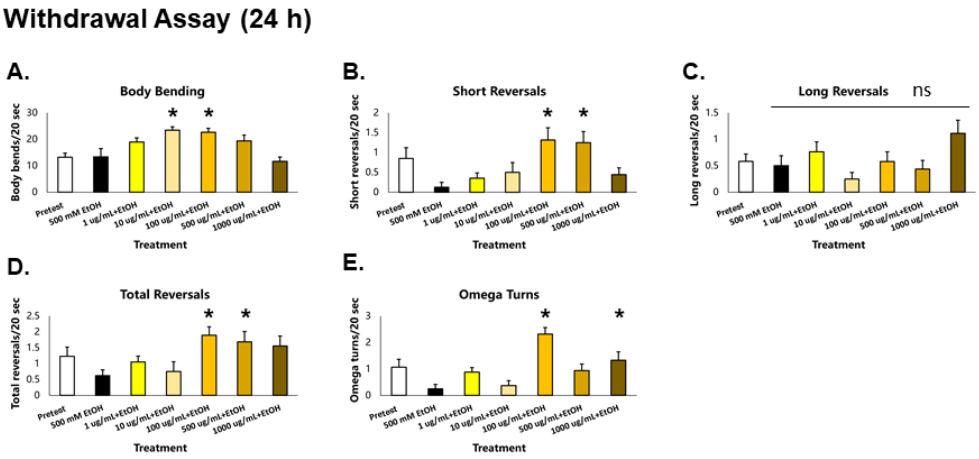

Figure 6. GRE protects locomotion from deficits induced by acute EtOH withdrawal after a 24 h exposure in C. elegans. Asterisks (*) denote significance at $p < 0.05$ versus vehicle.

Table 1. Coadministration of EtOH with GRE disrupts learned preference to EtOH in *C. elegans*.

GRE µg/ml	A (w/ EtOH)	B (w/o EtOH)	C (w/o EtOH)	D (w/ EtOH)	Total animals tested	PI
1	3	1	3	9	16	0.50
10	7	6	4	2	19	−0.05
100	3	6	3	2	14	−0.29
500	6	5	7	2	20	−0.20
1,000	1	10	3	3	17	−0.53
EtOH	2	5	3	6	16	0.00

yet to be induced in the worms, which is also consistent with a previous study in 2004 (Davies *et al*., 2004). All treatment groups were significantly higher than the control for plate tap and light touch to the head ($p < 0.01$). All treatment groups except for those given 10 µg/ml GRE were significantly higher than control in light touch tail and harsh touch responses ($p < 0.01$). Only the nose touch parameter was not significantly different from the control, which may suggest that the neurons providing this sensation, which are also responsible for sensing noxious chemicals and repellants (ASH), are unaffected by GRE. Nonetheless, GRE treatments significantly increased recovery of mechanosensation to an otherwise depressed worm given only EtOH.

Assessment of locomotion in ethanol-exposed *C. elegans* after stimulus withdrawal

When worms were withdrawn acutely (1 hour) from EtOH after prolonged exposure (24 hour), significant decreases in total reversals and omega turns were observed compared to preexposure conditions (white bar), indicating that withdrawal of EtOH results in deficits in foraging and roaming movements in *C. elegans* (Fig. 6D–E). Of the total reversals, short reversals were decreased, with no observable decreases in the long reversals (Fig. 6B–D). This implies that withdrawal also leads to incoordination between omega turns and long reversals—a pattern previously observed in the depression assay (Fig. 2D–E). Meanwhile, no decreases in body bending rate were seen (Fig. 6A), which was in contrast with a previous report showing reduction of crawl speed upon withdrawal (McIntire, 2010). Although this is the case, it is argued that the difference in effects with body bending is a function of exposure difference with previous studies. In this study, EtOH was applied directly to the worms after worm-picking; meanwhile, studies on alcohol withdrawal in *C. elegans* applied EtOH in the media and allowed for equilibration. The former method was carried out to ensure that exposure times among all worms were the same, which can be easily confounded by worm-picking.

Learned preference assay in *C. elegans*

In the learning preference assay, EtOH (500 mM) coupling with OP50 as a desirable stimulus after a minimum of 12 hours starvation led to an ethanol PI of zero. Compared with sodium acetate that is a natural chemoattractant to *C. elegans*, this indicates that the nematodes displayed equal preferences for sodium acetate and the previously neutral stimulus (EtOH), suggestive of a successful associative learning.

Meanwhile, exposure to GRE led to a dose-dependent decrease in preference to EtOH, with the greatest loss of preference observed with exposure to 1,000 µg/ml GRE (Table 1). At 1 µg/ml, the preference for EtOH increased twofold. Since GRE is a consistent acute depressant at high concentrations, the observed increase in EtOH preference may be due to suboptimal depression of excitatory neurons in the nematode, leading to rebound excitation that can in fact promote learning.

However, while GRE is effective at higher concentrations, acute lethality at 24 hours showed that GRE is most toxic at the highest concentration (1,000 µg/ml). While all other concentrations are relatively nontoxic at the threshold

survival rate of 85%, a question of benefits versus risks arises with GRE at higher doses, which may possibly be a concern in future work with higher mammalian models and in future therapy and management.

DISCUSSION

Caenorhabditis elegans as a model to explore ethanol-induced behaviors

Caenorhabditis elegans has proven to be a useful *in vivo* model for studies involving neurological conditions. While its nervous system is relatively simpler than most mammals, certain important features of the nervous system at the level of ion channels and neurotransmitters are conserved, such as gamma-aminobutyric acid (GABA) and acetylcholine and their receptors for inhibitory and excitatory neurotransmission (Risley *et al*., 2016). Other studies have also demonstrated that ethanol causes behavioral intoxication in *C. elegans* at tissue concentrations equivalent to that which causes intoxication in humans and other mammals (Davies *et al*., 2003). Still more recent are studies showing the ability of *C. elegans* to display withdrawal-related behavioral impairments after cessation of chronic ethanol exposure in terms of locomotion and chemotaxis (Scott *et al*., 2017). These studies on *C. elegans* make it a powerful model in investigating alcohol withdrawal and agents that could potentially address this, now made more relevant with today's major public health concern of alcohol dependence.

GRE as an acute depressant in *C. elegans*

In the nematode *C. elegans,* GABA receptors have a relatively conserved structure and function in affecting the trajectory of alcohol withdrawal. In fact, the most abundant synapses for inhibition in the worm model are those that use the neurotransmitter GABA (Lee *et al*., 2009). In this study, exposure to 500 mM EtOH, which is a known potentiator of GABA, led to an increase in meaningless long reversals, incoordination between long reversals and omega turns, and reduced general and nose touch sensations, which correlate with acute alcohol intoxication in humans. These point to the action of GRE as an acute depressant of locomotion and sensation, which is consistent with its traditional use as a *hangover* cure—a phenomenon tightly associated with withdrawal. However, the GABA receptor subunits UNC-49B/C of *C. elegans*, which share similarities with mammalian $GABA_A$ receptor subunits, are insensitive to benzodiazepines, precluding any comparisons of GRE with the first line (Bamber *et al*., 2003, 2005).

GRE increases tolerance to EtOH over time

In the study conducted, the definition of *acute alcohol tolerance* can be one or more of three: (1) the speed of recovery from intoxication, (2) neurochemical changes, and (3) neuronal adaptation (Davies *et al*., 2004; Jameson *et al*., 2018). However, due to the acuteness of exposure, tolerance in this study is likely to be due to (1) or (2).

At 40 minutes EtOH exposure, both locomotion and mechanosensation continued to be depressed, with the worms showing no signs of recovery. These observations are consistent with the findings of a previous study, where half of the exogenous ethanol accumulated in *C. elegans* internal tissues within 20 minutes of exposure. Hence, failure to induce tolerance after 40 minutes of ethanol exposure can be attributed to increased, albeit delayed, internal concentrations of ethanol (Alaimo *et al*., 2012). There are various candidate genes and proteins in the nematode model that can account for these results. *Caenorhabditis elegans* has been observed to develop ethanol tolerance and preference after continuous exposure to ethanol (Davies *et al*., 2004). The *npr-1* gene coding for the NPR-1 protein, a G protein-coupled receptor related to the mammalian neuropeptide Y receptors, has been found to negatively regulate the development of acute ethanol tolerance in *C. elegans* (Davies *et al*., 2004). Hence, inhibition of the NPR-1 receptor may be a plausible mechanism by which GRE can improve tolerance in *C. elegans*. On the other hand, enzymatic reactions pertaining to EtOH metabolism may also be affected, with a familiar target being alcohol dehydrogenase. Indeed, ADH in *C. elegans* has been directly demonstrated using a spectrophotometric assay.

Withdrawal-induced deficits in locomotion are rescued by GRE

The slo-1 allele encodes the BK potassium channel in humans and may have a central role in ethanol response. This large-conductance, calcium-gated potassium channel ethanol target in humans is conserved across species related by linear descent—such as man, *Drosophila melanogaster, C. elegans*, and *Mus musculus*—and produces withdrawal symptoms observable in the nematode (Mulholland *et al*., 2009; Scott *et al*., 2017). This channel has been identified as a major ethanol target in *C. elegans*, specifically for the ethanol effects on locomotion and egg-laying behavior. Indeed, activation of the BK potassium channel EtOH in *C. elegans* leads to inhibition of neuronal activity *in vivo*. Over the last decade, BK channels have emerged as important targets for the development of acute ethanol tolerance and for altering neuronal excitability following chronic ethanol consumption (Chick *et al*., 2000; Froehlich *et al*., 1990).

In this study, acute withdrawal of *C. elegans* from EtOH after prolonged (24 hours) exposure led to a decrease in short reversals and omega turns, with the sparing of long reversals and body bending. Meanwhile, coadministration of EtOH with GRE prior to withdrawal led to less prominent decreases in locomotion—suggesting a role of GRE in suppressing alcohol withdrawal. It may be possible that, apart from the acute depressing effects of GRE that continually tapers off rebound excitation from withdrawal, GRE also activates the BK channel expressed by the slo-1 gene, leading to decreased withdrawal behaviors. Indeed, the enhanced function of this channel has been demonstrated to drastically reduce withdrawal-induced behavioral impairments in *C. elegans* (Scott *et al*., 2017) and is therefore a possible target of GRE in protecting against withdrawal behavior.

GRE disrupts learned preference to EtOH *in vivo*

Alcohol is one of the 10 classes of drugs involved in substance-related disorders as mentioned in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders. These drugs directly trigger the brain's reward pathways instead of going through the process of adaptive behavior (American Psychiatric Association, 2013). Alcohol has been found to affect most neurotransmitters, but most prominently, it increases the activity of GABA and the receptors for N-methyl-D-aspartate (NMDA).

Upon acute alcohol intake, GABA production is increased, and NMDA receptors are inhibited postsynaptically. However, chronic alcohol consumption leads to the increased density of the GABA receptors while NMDA receptors are made more responsive. In addition to these two neurotransmitters, acute alcohol intake has also been found to increase dopamine levels in the brain. The increase in dopamine caused by the alcohol intake leads to alterations in the dopamine pathway that leads to the person craving more alcohol (Jameson *et al*., 2018).

In this study, pathways of alcohol dependence were partially demonstrated via associative learning of chemically neutral EtOH with a desired chemical stimulus (OP50). Whether it is an internal or an external cue being used to motivate learning, a reward response occurs once it is done. This response can be associated with the neurotransmitter dopamine. It is this same mechanism that makes the use of addictive substances, such as alcohol, rewarding (Hyman, 2005).

Relevant to this study was the finding that mutant nematodes deficient in dopamine and serotonin synthesis enzymes do not develop EtOH preference (Lee *et al*., 2009), which may point to its role on learned preference to EtOH. Hence, the significantly high disruption of learned preference by GRE coadministration at 1,000 µg/ml suggests that dopamine and serotonin are likely candidates as neurotransmitters affected by ginger exposure—a probability that warrants a quantitative investigation.

Phytochemicals found in ginger

Although the study did not employ a phytochemical analysis of compounds found in GRE, there is an abundance of data in the literature on its possible active compounds. Ginger is composed mostly of phenols (8.21%), followed by alkaloids, flavonoids, tannins, saponins, glycosides, and others (Alagbe *et al*., 2021). Of these, 6-gingerol has been shown to promote longevity and stress resistance in *C. elegans* (Lee *et al*., 2018), while 6-shogaol has been found to have the strongest inhibitory activity on serotonin 5-HT receptors for its antiemetic activity (Mao *et al*., 2019). Further investigation using bioassay-guided purification or behavioral tests using pure compounds is warranted to determine the antiaddictive compounds in ginger.

CONCLUSION

In this study, we show that *C. elegans* appropriately models alcohol intoxication, tolerance, withdrawal, and preference with similar presentations in higher animal models. Furthermore, we show that GRE is a short-acting depressant, which also improves tolerance and protects against aberrations caused by EtOH withdrawal after prolonged exposure. Lastly, GRE disrupted the learned preference of *C. elegans* for EtOH, reducing it by as much as 53% at 1,000 µg/ml. Taken together, these results validate the traditional use of ginger as a hangover cure and may warrant further investigation on its potential for alcohol-related emergencies and dependencies.

ACKNOWLEDGMENTS

The authors would like to thank their advisers from the Department of Pharmacology and Toxicology, Dr. Richard Henry Tiongco III and Dr. Jaime Purificacion, for providing inputs in the design of the study. Their colleagues from the *C. elegans* team are also acknowledged: Ma. Jamela Lacuban, Francesca Marie Lagrosa, Liza Marie Laroco, Kristel Joy Limjuco, Francis Martin Logmao, Melissa Magad, Jose Marcel Magno, Danee Coline Mangila, and Jacinto Armando Mantaring.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

FUNDING

This study was partially funded by the National Institutes of Health of the University of the Philippines Manila (NIH-2018-045). Further, all strains were obtained from and provided by the Caenorhabditis Genetics Centre (CGC) of the University of Minnesota, which is funded by the NIH Office of Research Infrastructure Programmes (P40OD010440).

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design of the work, the acquisition, analysis, and interpretation of data, the drafting of the work and its critical revision for important intellectual content, and the final approval of the version to be published, have agreed to be accountable for all aspects of the work, and also contributed to ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. All are eligible to become authors based on the criteria of the International Committee of Medical Journal Editors (ICMJE).

REFERENCES

Alagbe JO, Oluwafemi RA, Omolade LA, Adetope AS. Effects of dietary inclusion of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) oil (GGO) mixtures on carcass characteristics and sensory evaluation of broiler chickens. Int J Pure Appl Zool, 2021; 9(8):15–20.

Alaimo JT, Davis SJ, Song SS, Burnette CR, Grotewiel M, Shelton KL, Pierce-Shimomura JT, Davies AG, Bettinger JC. Ethanol metabolism and osmolarity modify behavioral responses to ethanol in *C. elegans*. Alcohol Clin Exp Res, 2012; 36(11):1840–50.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th edition, American Psychiatric Publishing, Arlington, VA, 2013.

Bamber BA, Twyman RE, Jorgensen EM. Pharmacological characterization of the homomeric and heteromeric UNC-49 GABA receptors in *C. elegans*. Br J Pharmacol, 2003; 138(5):883–93.

Bamber BA, Richmond JE, Jorgensen EM. The composition of the GABA receptor at the *Caenorhabditis elegans* neuromuscular junction. Br J Pharmacol, 2005; 144(4):502–9.

Chick J, Anton R, Checinski K, Croop R, Drummond DC, Farmer R, Labriola D, Marshall J, Moncrieff J, Morgan MY, Peters T, Ritson B. A multicentre, randomized, double-blind, placebo-controlled trial of naltrexone in the treatment of alcohol dependence or abuse. Alcohol Alcohol, 2000; 35:587–93.

Davies AG. Pierce-Shimomura JT, Kim H, VanHoven MK, Thiele TR, Bonci A, Bargmann CI, McIntire SL. A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans*. Cell, 2003; 115(6):655–66.

Davies AG, Bettinger JC, Thiele TR, Judy ME, McIntire SL. Natural variation in the npr-1 gene modifies ethanol responses of wild strains of C. elegans. Neuron, 2004; 42(5):731–43.

Dumville JC, Torgerson DJ, Hewitt CE. Reporting attrition in randomised controlled trials. BMJ, 2006; 332(7547):969–71.

Ernst E, Pittler MH. Efficacy of ginger for nausea and vomiting: a systematic review of randomized clinical trials. Br J Anaesth, 2000; 84(3):367–71.

Froehlich JC, Harts J, Lumeng L, Li TK. Naloxone attenuates voluntary ethanol intake in rats selectively bred for high ethanol preference. Pharmacol Biochem Behav, 1990; 35:385–90.

Goodman MB. Mechanosensation. In: WormBook (ed.). The *C. elegans* research community, WormBook, 2006; Pasadena: CA. doi:10.1895/wormbook.1.62.1.

Hyman SE. Addiction: a disease of learning and memory. Am J Pysch, 2005; 162(8):1414–22.

Irving A, Goodacre S, Blake J, Allen D, Moore SC. Managing alcohol-related attendances in emergency care: can diversion to bespoke services lessen the burden? J Emerg Med, 2017; 35(2):79–82.

Jameson JL, Kasper KD, Longo DL, Fauci AS, Hauser SL, Loscalzo J. Harrison's principles of internal medicine. 20th edition, McGraw-Hill Education, Ney York, NY, 2018.

Katiki LM, Ferreira JF, Zajac AM, Masler C, Lindsay DS, Chagas AC, Amarante AF. *Caenorhabditis elegans* as a model to screen plant extracts and compounds as natural anthelminthics for veterinary use. Vet Parasitol, 2011; 182(2-4):264–8.

Lee J, Jee C, McIntire SL. Ethanol preference in *C. elegans.* Genes Brain Behav, 2009; 8(6):578–85.

Lee EB, Kim JH, An CW, Kim YJ, Noh YJ, Kim SJ, Kim JE, Shrestha AC, Ham HN, Leem JY, Jo HK, Kim DS, Moon KH, Lee JH, Jeong KO, Kim DK. Longevity and stress resistant property of 6-gingerol from *Zingiber officinale* Roscoe in *Caenorhabditis elegans.* Biomol Ther, 2018; 26(6):568–75.

Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, Li HB. Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). Foods, 2019; 8(6):185.

McIntire, SL. Ethanol. In: WormBook (ed.). The *C. elegans* research community, WormBook, 2010; Pasadena: CA. doi:10.1895/wormbook.1.40.

Mulholland PJ, Hopf FW, Bukiya AN, Martin GE, Liu J, Dopico AM, Bonci A, Treistman SN, Chandler LJ. Sizing up ethanol-induced plasticity: the role of small and large conductance calcium-activated potassium channels. Alcohol Clin Exp Res, 2009; 33:1125–35.

Olson KN, Smith SW, Kloss JS, Ho JD, Apple FS. Relationship between blood alcohol concentration and observable symptoms of intoxication in patients presenting to an emergency department. Alcohol Alcohol, 2013; 48(4):386–9.

World Health Organization. Alcohol: Philippines, 2014. Available via http://www.who.int/substance_abuse/publications/global_alcohol_report/profiles/phl.pdf (Accessed on 19 September 2018).

Penning R, van Nuland M, Fliervoet LA, Olivier B, Verster JC. The pathology of alcohol hangover. Curr Drug Abuse Rev, 2010; 3(2): 68–75.

Risley MG, Kelly SP, Jia K, Grill B, Dawson-Scully K. Modulating behavior in *C. elegans* using electroshock and antiepileptic drugs. Plos One, 2016; 11(9):e0163786.

Scott LL, Davis SJ, Yen RC, Ordemann GJ, Nordquist SK, Bannai D, Pierce JT. Behavioral deficits following withdrawal from chronic ethanol are influenced by SLO channel function in *Caenorhabditis elegans*. Genetics, 2017; 206(3):1445–58.

Torkzadeh-Mahani S, Nasri S, Esmaeli-Mahani S. Ginger (*Zingiber officinale* Roscoe) prevents morphine-induced addictive behaviors in conditioned place preference tests in rats. Addict Health, 2014; 6(1-2):65–72.

Williams PL, Dusenbery DB. Using the nematode *Caenorhabditis elegans* to predict mammalian acute lethality to metallic salts. Toxicol Ind Health, 1988; 4(4):469–78.

Zhao B, Khare P, Feldman L, Dent JA. Reversal frequency in *Caenorhabditis elegans* represents an integrated response to the state of the animal and its environment. J Neurosci, 2003; 23(12):5319–28.

How to cite this article:
Manalo RVM, Lapuz BLC, Lim KK, Legaspi KEY, Lota PMM, Lucas FMB, Maldisa AN, Malipot VC, Lo CBO, Lichauco AKP, Medina PMB. The effects of ginger (*Zingiber officinale*) rhizome extract on ethanol-induced behaviors in *C. elegans*. J Appl Pharm Sci, 2022; 12(05):187–195.