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Research Article

Potential effect of *Escherichia coli* Shiga toxin metabolites in the induction of cognitive dysfunction and stress memory formation in naïve goldfish *Carassius auratus*

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Abstract
The oral-gut-brain (OGB) axis is a three-way communication process that forms cognitive functions, especially learning and memory (LM) formation. Recent studies showed that the OGB axis is pivotal in efficiently forming and regulating the brain homeostasis mechanism. In this OGB axis, oral and gut commensals play a major role in the bidirectional communication with the brain through the blood-brain barrier (BBB). The dysbiosis of oral and gut commensals results in cognitive dysfunctions like cognitive decline. The present study aimed to study the effect of *Escherichia coli* induced oral-gut dysbiosis on cognitive memory (CM) formation with the help of a cue-based learning paradigm (CBLP). Other than CM formation, stress memory (SM) formation was also studied with the help of behavioural paradigms like predator exposure test (PET), light and dark box test (LDBT), and open field test (OFT) in naïve goldfish *Carassius auratus*. The results of the study proved that the OGB axis is possibly involved in the formation of CM by inhibiting stress formation in a habituated, serene environment. Behavioural responses of *E. coli*-infused groups showed that higher colonization/accumulation of *E. coli* results in the formation of cognitive decline through the release of shiga toxin (ST) metabolites. It also showed that the release of ST metabolites may disrupt gut dysbiosis through the development of gastroenteritis. Developed gastroenteritis further results in cognitive memory - decline (70 -75 %) due to the long-term existence of *E. coli*-induced oral/gut dysbiosis. Thus, the present study stated the possible effect of *E. coli* shiga toxin metabolites on the development of cognitive dysfunction in a unique manner.

Keywords: Cognition, *Escherichia coli*, Oral-gut-brain axis, Shiga toxin metabolites, Stress memory

INTRODUCTION

The oral-gut-brain (OGB) axis includes the anatomically connected structure of the oral cavity (OC) and gut (G) with the brain. In this OGB axis, OC and G consist of rich and ecologically diverse microorganisms in beneficial and pathogenic forms (Tortora *et al.,* 2023; Sansores-España *et al.,* 2021). In normal conditions, the rich diversity of resident beneficial microorganisms (commensal) takes control or inhibits pathogenic colonization (PC) in OC and G. Residential beneficial microorganisms, including *Lactobacillus* species carry out

this pathogenic control. *Lactobacillus* species like *Lactobacillus acidophilus, Lactobacillus plantarum,* and *Lactobacillus rhamnosus* may effectively reduce the colonization of PC against experimental animal models like *Carassius auratus* (Aggarwal *et al.,* 2023; Squarzanti *et al.,* 2024; Mukilan *et al.,* 2024b). Inhibited PC develops proper cognitive learning and memory (LM) formation against an exposed stimulus through specific neurotransmitter production. This neurotransmitter production is carried out in the presynaptic neuron with the help of precursor neurochemicals (PN) from the gut through the blood-brain barrier (BBB) in the experimental mouse models (Dicks, 2022; Amartumur *et al.,* 2024; Mukilan *et al.,* 2024a).

Productions of neurotransmitters result in the activation and regulation of postsynaptic neuronal receptors and neuronal signaling molecules [cyclic adenosine monophosphate (cAMP), and protein kinase A (PKA)] through the release of neurotransmitters into the synaptic cleft (Ganesh *et al.,* 2010; Chen *et al.,* 2021; Mukilan, 2025a). The regulation of cAMP and PKA results in the active phosphorylation of cAMP response element binding protein (CREB). The CREB phosphorylation further induces immediate early genes (IEGs), and postsynaptic density proteins (PSDs) expressions. This activity-dependent expression of neuronal signaling molecules (NSM) results in the development of long-term memory formation (LTMF) in the indian short-nosed fruit bat *Cynopterus sphinx*, and naïve goldfish *C. auratus* (Ganesh *et al.,* 2012; Mukilan *et al.,* 2018a; Thangaleela *et al.,* 2018; Rajan, 2021; Mukilan *et al.,* 2024c). Other than listed NSM, some microRNA (miR) like miR – 132 and 148a also act as a positive and negative regulator of LTMF in a normal condition. This LTMF process includes exposure to an unexposed stimulus (exploration), acquaintance with new information (training), and memory reconsolidation (testing) sequentially in young experimental models of *C. auratus*, *C. sphinx*, and Wistar rats (Mukilan *et al.,* 2015; Rashidi *et al.,* 2023; Mukilan, 2024a).

The sequential phases of LTMF may be hindered due to pathogenic colonization during diseased conditions. Formed disease state initially results in the induction of oral dysbiosis (OD) through the reduction of oral beneficial flora (OBF). The reduction of OBF may happen due to the increased level of pathogenic microorganisms, which includes periodontal (P) and non-periodontal (NP) microorganisms in the OC of *Mus musculus* (Georges *et al.,* 2022; Mukilan, 2023; Elghannam *et al.,* 2024). Colonization and multiplication of P and NP pathogens result in the formation of poor oral hygiene (POH), biofilm formation (BF), and LPS production (LPSP) within the surface of OC. Produced LPS further transmitted from the OC to the gut through the mucus membrane (Mukilan, 2022; Said-Sadier *et al.,* 2023; Li *et al.,* 2024). Once reaching the gut, transmitted LPS further destroys the commensals present in the gut and forms gut dysbiosis (GD). The formed GD later on results in the decreased production and transmission of PN to the brain through the BBB. The reduced transmission of these PN further results in the formation of cognitive dysfunction in various brain regions. Cognitive dysfunctions later make an imbalance in the brain homeostasis mechanism in a time-dependent manner based on the studies carried out on the rodent models and randomized human trials (Anand *et al.,* 2023; Shahbazi *et al.,* 2023; Mukilan *et al.,* 2025b). Based on recent research studies, the present study aimed to identify the effect of *Escherichia coli* colonization in the induction of oral and gut dysbiosis through the administration of *E. coli* in varying concentrations. It also studied the effect of *E. coli* metabolite (shiga toxin) in the formation of cognitive dysfunctions with the help of a cue-based learning paradigm (CBLP), predator exposure test (PET), light and dark box test (LDBT), and open field test (OFT) using adult naïve goldfish *Carrasius auratus*.

MATERIALS AND METHODS

Experimental fishes

Commercially available aquarium-bred adult naïve goldfish (*Carassius auratus*) having a body weight of 6-14 g, body length of 6.5 – 7.5 cm, and both sexes (male and females) were procured from a local aquarium situated in P.N. Palayam, Coimbatore. The purchased fishes were transported to the laboratory aquarium in an amiable condition without any transportation stress. Once reaching the laboratory conditions, experimental fishes (n = 30) were housed in standard glass rectangular tanks (SGRT) having a size of 42 X 30 X 21 (length, breadth, and height) inches after confirming the absence of microbial colonization in the skin. The experimental fishes were allowed to stay in the SGRT for seven days to adapt (assimilate) to the laboratory conditions. During the assimilation process, experimental fishes were provided with ambient temperature (24 ± 2 ºC), light and dark cycle (14: 10 hours), and continuous air circulation (24 X 7). Experimental fishes fed with commercial dry round (CDR) food pellet (Taiyo Pet Products Pvt Ltd, India) thrice a day (9.00, 13.00, and 18.00 h). The water quality of the SGRT was maintained by continuous filtering with the help of an internal debris filter. The internal debris filter was cleaned between alternative days to maintain dissolved oxygen (DO) content and turbidity in the laboratory aquarium water. To maintain DO content, existing aquarium water was replaced two times a week based on the aquarium water quality (Mukilan *et al.,* 2025c).

Animal Ethics approval

The Institutional animal care guidelines of Sri Ramakrishna Group of Institutions (SRGI), Coimbatore, Tamil Nadu, India, were followed for the Performed behav-

ioural paradigms and study designs.

Experimental study design

Cue-based reward learning paradigm

Experimental fishes received a cue-based reward learning paradigm (CRLP) in an SGRT [42 (length) X 30 (breadth) X 21 (height) inches], which consisted of three different compartments – two feeding chambers (FC) (left chamber [LC, 6 (length) X 5 (breadth) X 21 (height) inches], and right chamber [RC, 6 (length) X 5 (breadth) X 21 (height) inches]) and one central chamber (CC, 30 (length) X 20 (breadth) X 21 (height) inches). The central opening in the LC and RC ensured the proper movement of experimental fish into FC from the CC. Both the FC acted as a positive/negative reward chamber with the blue/red color cues. The CDR food pellets were given as a food reward for the correct responses during the CRLP (Mukilan, 2024b).

Predator exposure test

Predator exposure test (PET) was carried out in an SGRT [42 (length) X 30 (breadth) X 21 (height) inches], having three different zones – no fear (NF) zone, mid-fear (MF) zone, complete fear (CF) zone with a length, breadth, and height of 14 X 10 X 21 inches. The central opening in the NF and CF zones connects all three zones in the SGRT and one individual closed compartment (ICC) within the NF zone. The ICC was used to keep their predator (bluegills) inside the NF zone and study the fear memory formation in the experimental fishes.

Light and dark box test

Light and dark box test (LDBT) was performed in an SGRT [42 (length) X 30 (breadth) X 21 (height) inches], having two different compartments – bright compartment (BC) and dark compartment (DC). Both the compartments have a size of [21 (length) X 15 (breadth) X 21 (height) inches] and are differentiated based on the availability of photoperiod (light or dark). The DC was separated from BC by sticking a black adhesive film on the separated surface. The designed LDBT apparatus was used to study the formation of anxiety-like behavior in experimental fishes.

Open-field test

An open-field test (OFT) was performed to identify the effect of anxiety-like behaviour on the motor activity of experimental fishes. The OFT was performed in an SGRT [42 (length) X 30 (breadth) X 21 (height) inches] having equal-sized box diagrams (10 X 5 cm) drawn at the bottom. Following LDBT, experimental fishes were introduced into the behavioural apparatus to identify their mobility in the inner and outer compartments. Motor activities of the experimental fishes were calculated based on the time spent in the inner compartment (TIC), and time spent in the outer compartment (TOC).

Culture acquaintance, purity confirmation, and oral infusion mixture preparation

E. coli culture (isolated from feces) used in this study was availed from the PSG Institute of Medical Sciences & Research (PSG IMSR), Peelamedu, Coimbatore, Tamil Nadu, India, as a quadrant streaked plate. Availed culture was initially grown on a nutrient broth medium for 12-24 hours to confirm its growth parameters (GP). Followed by the confirmation of GP, a loopful of overnight grown culture was used to prepare quadrant streaked nutrient agar plates (QSNAP). Prepared QSNAP incubated at 37 ºC for 12 hours to form individual colonies. After purity confirmation, individual colonies were used to prepare the oral infusion mixture (OIM) by arousing the overnight culture. Grown overnight cultures were used to prepare OIM with sterile phosphate buffer saline (PBS) in varying ratios of 20:80, 40:60, 60:40, and 80:20.

Experimental study groups

Experimental study groups (ESGs) were divided into two major categories based on OIM's non-receivable/ receivable state. Experimental fishes that received OIM were categorized as experimental infusive groups (EIG). Further, these EIGs were differentiated into four different groups i.e. EIG–1, EIG–2, EIG–3, and EIG–4 based on the receivable ratios of 20:80, 40:60, 60:40, and 80:20. To study the effect of OIM, one experimental non-infusive group (ENG) was used as standard control. The standard control ENG mimicked the development of cognitive function in a normal state.

Statistical representation

Behavioural scores of the CBLP, PET, LDBT, and OFT were used to prepare bar diagrams with the help of average, standard error values using a Microsoft excel program.

RESULTS

Role of *Escherichia coli* oral microbial infusions in the development of neuroprotective and cognitive dysfunctions

Initially, the purity of the obtained *E. coli* culture was confirmed by the quadrant streak plate method. Individual *E. coli* colonies isolated from the quadrant plate were used for the preparation of OIM and also stored in the form of a simple streak plate method (Fig. 1). To identify the dual role of *E. coli* in the development of neuroprotective and cognitive dysfunctions, a CBLP was used in the study. In this CBLP, one ENG and four EIGs (EIG– 1, 2, 3, and 4) were used to study the impact of *E. coli* OIM (varying ratios) in standard laboratory conditions with the use of naïve goldfish *C. auratus*. Four different phases used in the CBLP include habituation, exploration, training, and testing. Initially, all ESGs (ENG and EIG) were habituated in the laboratory

Fig. 1. *Representative plate pictures showing the purity of E. coli culture used to prepare oral microbial infusions (OMI) in the present study*

aquarium for seven days (days 1 – 7) for their adaptation to the laboratory environment. Following adaptation, all ESGs were allowed to explore the behavioural experimental setup during the exploration phase between days 8 – 10 for 15 minutes/day. Behavioural responses of the exploration phase showed that all animals were active and actively engaged in exploring all three chambers (LC, CC, and RC) (Fig. 2). After completion of the exploration phase, EIGs received *E. coli* OIM in a ratio of 20:80 (EIG - 1), 40:60 (EIG - 2), 60:40 (EIG - 3), and 80:20 (EIG - 4) on day 11. After receiving *E. coli* OIM, EIGs were maintained in different habituation tanks for seven days (days 12-18) for their settlement towards the gut. Following the exploration phase, both ENG and EIGs were allowed to perform the CBLP training phase between days 19-21 for 15 minutes/day. Behavioural responses of the ENG and EIGs were calculated based on the time spent in CC, LC, and RC for 900 seconds. On day 19, most of the EIG and ENG spent more time in the CC than the LC/RC. From days 20 and 21, the amount of time spent in CC was gradually reduced, and time spent in LC and RC increased. The spent time increase in LC and RC showed that the animal tried to learn the positive or negative color cue within the provided time (900 seconds). Increased responses to the FCs result in the identification of food rewards based on the correct responses. Obtained behavioural responses showed that OIM never had an impact on the learning abilities of EIGs in lower ratios; however, it had a mild impact on higher ratios compared to ENG (Fig. 3). At the end of the training, three days time gap (days 22-24) was provided for the formation of memory through memory consolidation process in the ESGs.

Following CBLP training, testing was carried out for all ESGs, including ENG and EIGs, to determine the impact of varying OIM ratios on memory reconsolidation. The CBLP testing phase was carried out between 25-27 days to identify the amount of time spent in CC, LC, and RC. The outcome of the behavioural testing scores showed that EIGs showed a gradual decrease in the number of attempts to the positive chamber (RC) compared to the ENG. The decline in the number of correct responses showed that OIM had an impact on the memory reconsolidation process through the formation of oral/gut dysbiosis (Fig. 4). Formed OD shows re-

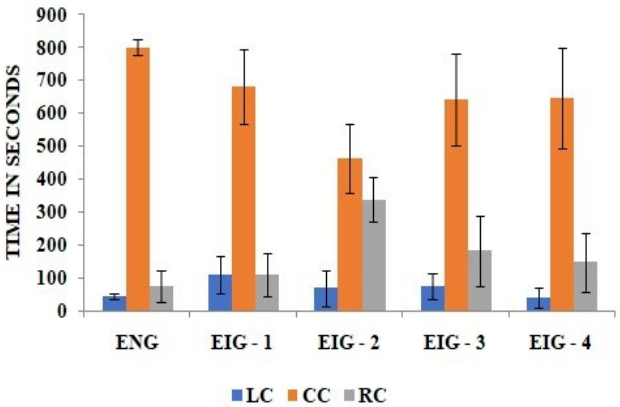

Fig. 2. *Behavioural responses of the exploration phase showed that all experimental study groups (ESGs) explored all three chambers (left chamber – LC, central chamber – CC, and right chamber – RC) in an active manner, which includes experimental non-infusive group (ENG) and experimental infusive groups (EIGs – EIG – 1, 2, 3, and 4)*

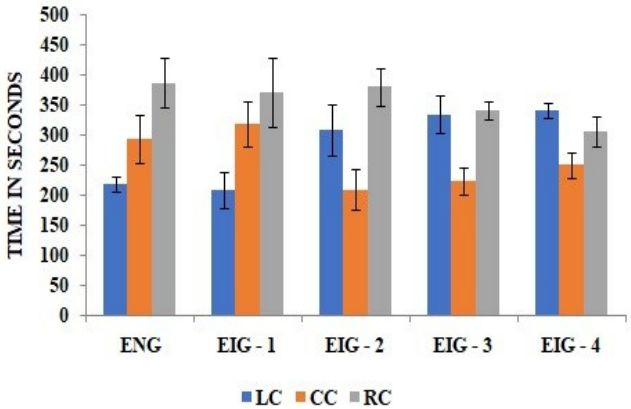

Fig. 3. *Behavioural responses of the CBLP training phase showed that both experimental non-infusive group (ENG) and experimental infusive groups (EIGs – EIG – 1, 2, 3, and 4) responded to positive stimuli through the increased number of visits to right chamber (RC) compared to number of visits to left chamber (LC) central chamber – CC, and right chamber (RC)*

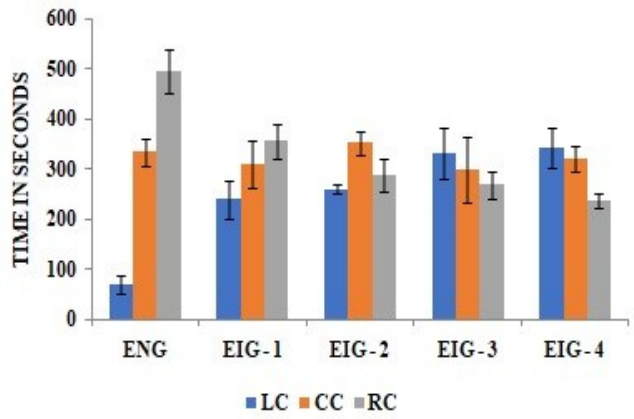

Fig. 4. *Behavioural responses of the CBLP testing phase showed that experimental infusive groups (EIGs – EIG – 1, 2, 3, and 4) responded to positive stimuli through the decreased number of visits to the right chamber (RC) compared to experimental non-infusive group (ENG). It also showed that E. coli oral microbial infusions had an impact on the development of cognitive memory decline in an increased manner (LC – left chamber, CC – central chamber*)

duced transmission of PN from the gut, which may result in the formation of cognitive decline through reduced neurotransmitter production in the brain of naïve goldfish *C. auratus*. Along with PN, some infectious metabolites are also transported to the brain, which induces inflammatory reactions through reactive oxygen species (ROS) production in the hippocampal brain regions. Induced neuroinflammatory reactions also join hands with the reduced neurotransmitters in the formation of cognitive decline in *C. auratus*. Comparative analysis of CBLP training and testing responses showed that OIM had an impact on the retrieval of stored information through the memory reconsolidation process. It also proved that lower OIM ratios greatly impacted cognitive memory formation through its neuroprotective effect. However, OIM higher ratios may develop cognitive dysfunction through oral/gut dysbiosis and brain neuroinflammation in naïve goldfish *C. auratus* (Fig. 5).

Effect of *Escherichia coli* oral microbial infusions in the anxiety-like behavior development

For the identification of anxiety-like behaviour (ALB) formation, OFT and LDBT were performed after the completion of CBLP training and testing phases. Initially, all ESGs were allowed to perform OFT between days 22-24 to identify the effect of OIM on the motor behaviour of the experimental fishes. The OFT behavioural responses showed that OIM never affected the motor behaviour of ESGs. Outcome results also proved that the amount of time spent in the inner compartment (TSI) is high in the EIG– 1, and EIG– 2 compared to the other two infusive groups (EIG– 3 and EIG – 4). However, the observed time spent is vice-versa in EIG – 3 and EIG – 4, with an increasing amount of time spent in the outer compartment (TSO). Observed results showed that OIM did not affect the motor behaviour of the ESGs, which developed a mild level of ALB in EIG – 3 and EIG – 4. Thus the observed OFT responses showed that OIM had a mild effect on the development of ALB after the completion of CBLP training (Fig. 6).

To identify the further effect of OIM on ALB, a LDBT was performed by both ENG and EIGs between days 28-30. After completion of the CBLP testing phase, all ESGs were allowed to perform LDBT in a photo-light controlled laboratory environment to identify the level of ALB formation. Observed LDBT responses showed an increased amount of time spent in DC compared to the LC in EIGs compared to the ENG. The ENG showed a higher amount of time spent in the LC compared to the DC. Observed time spent in the DC showed the development of ALB in EIGs compared to the ENGs (Fig. 7). Comparative analysis of OFT and LDBT showed that OIM had an effect on the development of ALB in the

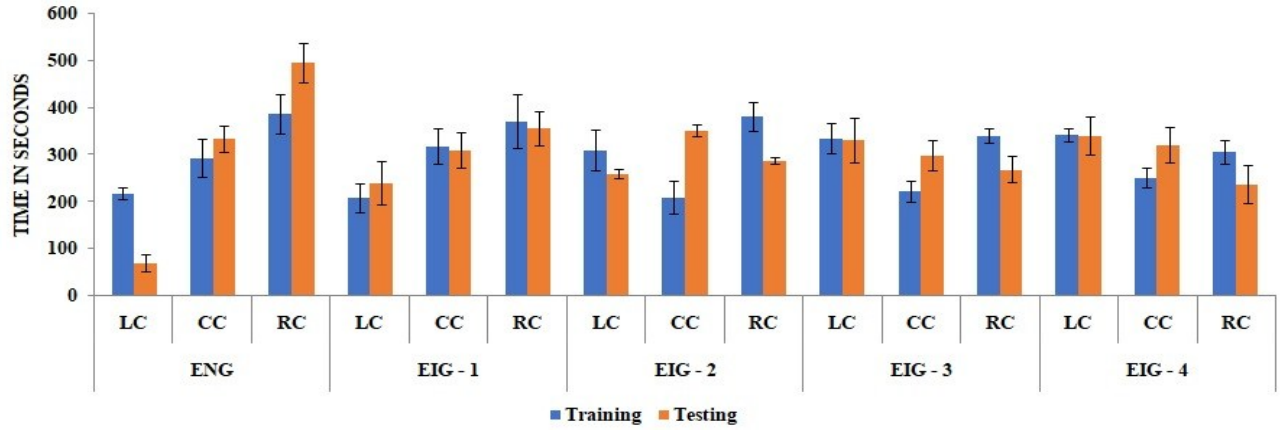

Fig. 5. *Comparative analysis of the CBLP training and testing phase showed that experimental non infusive groups (ENG) and experimental infusive groups (EIGs – EIG – 1, 2, 3, and 4) responded to positive and negative stimuli. It also showed that E. coli oral microbial infusions had an impact on the development of cognitive memory decline in a concentration-dependent manner (LC – left chamber, CC – central chamber, RC – right chamber)*

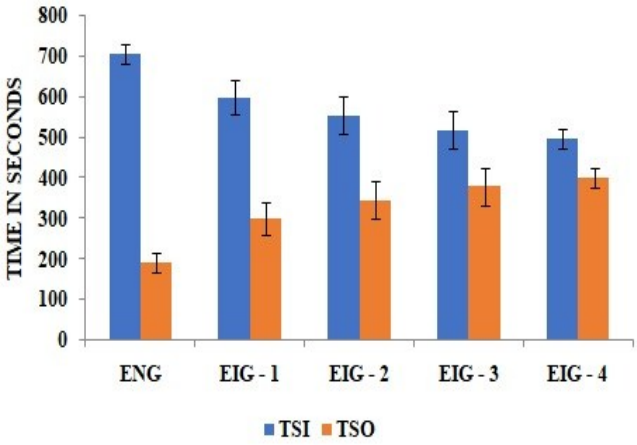

Fig. 6. *Open-field test (OFT) responses showed that E. coli oral infusion mixtures (OIM) did not affect the motor behavior of experimental study groups (ESGs). Behavioural scores of OFT showed the development of anxiety-like behavior in the experimental infusive groups (EIGs) by the amount of time spent in the outer compartment (TSO) compared to the amount of time spent in the inner compartment (TSI) (ENG – experimental non infusive group)*

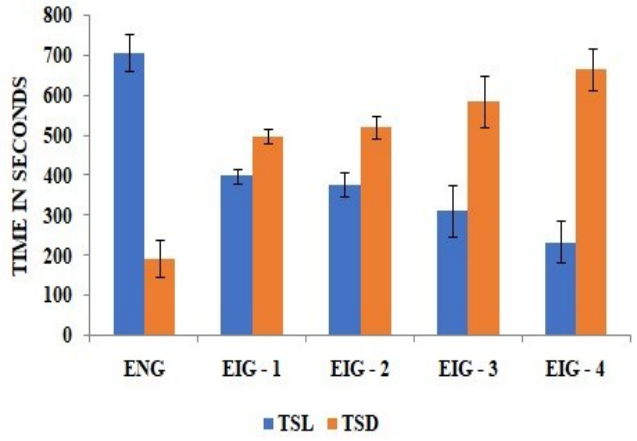

Fig. 7. *Behavioural responses of Light and dark box test (LDBT) showed the formation of anxiety-like behavior (ALB) in experimental infusive groups (EIGs) compared to experimental non infusive group (ENG) (TSL – Time spent in light chamber, TSD – time spent in dark chamber)*

EIGs compared to the non-infusive ENGs. It also showed that the formation of ALB is initiated during the period of CBLP training and established during the CBLP testing phase. Thus, the development of ALB plays a pivotal role in the formation of cognitive dysfunctions in naïve goldfish *C. auratus* (Fig. 8).

Impact of *Escherichia coli* oral microbial infusions in stress memory formation

Finally, the impact of OIM on stress memory (SM) formation was analyzed with the help of PET between days 31-33. In PET, all ESGs were allowed to explore all three zones (NFZ, MFZ, and CFZ) for 15 minutes/day. The amount of time spent in NFZ, MFZ, and CFZ was recorded in seconds during the behavioural analysis in SGRT. Observed behavioural responses showed that ENG spent more time in NFZ, which shows the absence of SM in the non-infusive group. The observed behavioural response was vice-versa in infusive groups like EIG – 1, EIG – 2, EIG – 3, and EIG – 4. The EIGs (EIG– 1, EIG– 2) spent more time in MFZ, and less time in CFZ compared to EIG– 3 and EIG– 4. The increased amount of time spent in MFZ showed the lower level of SM formation in EIG– 1 and EIG– 2. The lower ratios of OIM showed a moderate level of SM formation compared to the non-infusive group (ENG). Compared to EIG – 1 and EIG – 2, higher ratios of OIM had a higher level of SM formation, resulting in cognitive decline. Thus, the observed behavioural responses proved that OIM results in the development of SM based on the infused ratios in naïve goldfish *C. auratus* (Fig. 9).

DISCUSSION

The present study showed the effect of *E. coli* oral microbial infusions (EOI) on the formation of cognitive memory, stress memory, and anxiety-like behaviour with the help of behavioural paradigms like CBLP, PET, LDBT, and OFT. At first, OIM was prepared in four different ratios (20:80, 40:60, 60:40, 80:20) using freshly prepared sterile phosphate buffer saline (PBS) solution. The prepared formulations were infused into their respective EIGs with the help of an oral-guage. Outcome responses of the CBLP training phase showed that EIGs showed a mild reduction in learning abilities compared to the ENG – 1. It also stated that the lower ratios (20:80, 40:60) of EOI never impacted the development of cognitive decline compared to the higher (60:40, 80:20) ones. Overall, the training responses showed that higher ratios of EOI had a mild impact on learning abilities and a huge effect on initiating cognitive dysfunctions. Recent studies also reported that reduction in gut commensals (*Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *Pedicoccus*, *Enterobacter*, and *Clostridium*) may result in the initiation of cognitive dysfunctions (Mukilan, 2022; Piccioni *et al.,* 2023; Mukilan *et al.,* 2024b). The above-listed commensals produce acetic acid, lactic acid, hydrogen peroxide, and immunoglobuins (Ig) to control the colonization of *E. coli* in the gut. The prevention of gut *E. coli* colonization may result in the inhibited shiga toxin metabolite production at higher level during the training period which was supported by recent research findings in the field of OGB axis (Lee *et al.,* 2021; Heravi and Hu, 2023; Pitchaikani et al. 2024). Thus, the observed results showed that OIM had a mild effect on the learning abilities of experimental fishes.

Followed by CBLP training, CBLP testing responses showed that the OIM had a strong decline in the development of memory formation. Obtained results showed that OIM had an increasing impact on the memory re-

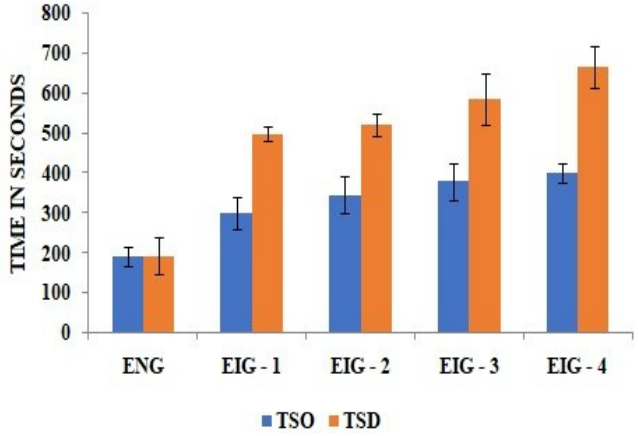

Fig. 8. *Comparative analysis of open field test (OFT) and light and dark box test (LDBT) showed the effect of E. coli OIM in the formation of anxiety-like behavior (ALB) in experimental infusive groups (EIGs). Observed time spent in the dark chamber (TSD) shows the interrelationship present between anxiety-like behavior and cognitive decline (ENG – experimental non infusive group, TSO - time spent in the outer compartment)*

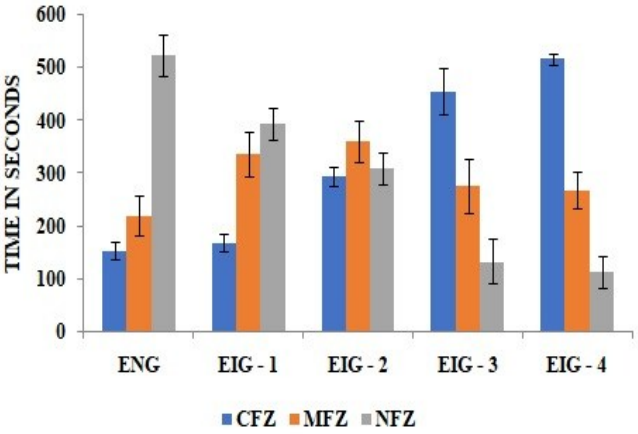

Fig. 9. *Behavioural responses of the predator exposure test (PET) showed the effect of stress memory formation in the E.coli infused experimental infusive groups (EIGs – 1, 2, 3, and 4) compared to experimental non infusive group (ENG) (CFZ – complete fear zone; MFZ – Mid fear zone, NFZ – no fear zone)*

consolidation (MR) process through the formation of oral and gut dysbiosis. In the initial condition, the progression of dysbiosis resulted in the reduction of beneficial microorganisms in the gut. The reduction of beneficial flora indirectly showed the colonization and multiplication of *E. coli* in the gut. Increased *E. coli* colonization may result in the production of shiga toxin, which has an impact on the formation of impaired cognitive memory formation (Kim *et al.,* 2020; Anjana and Tiwari, 2022; Schütze *et al.,* 2022; Kolobaric *et al.,* 2024). The formed cognitive impairment shows the reduced synthesis and transport of PN to the brain through the BBB. Reduced PN is further involved in producing the neurotransmitter in the least quantities. The least amount of neurotransmitters, later on, are involved in the induction of partial calcium influx, which results in the downregulation of neuronal signaling molecules involved in impaired LTMF in vertebrate animal models (Mukilan *et al.,* 2018b; Noe *et al.,* 2020; Morozova *et al.,* 2022). Later, impaired LTMF shows reduced RNA-dependent protein synthesis (RPS) within the neuronal connections. As a result of RPS, synaptic plasticity changes may happen in an inappropriate form, affecting the MR process in *C. auratus* (Monday *et al.,* 2022; Mukilan, 2023).

Impairment in the MR process results in the blockade of information transmission (IT) between the brain neurons. Blockade of IT also shows the inhibited/minimal level of commensals in the gut. During dysbiosis, a higher level of *E. coli* infection results in the inhibition of beneficial microorganisms and it may produce a least/reduced amount of PN as it becomes minor flora in the gut. Thus, the overload of *E. coli* colonies in the OC and G may produce shiga toxins. Later on, produced shiga toxin metabolites also activate the negative regulator (hypothalamus-pituitary (HP) axis) of cognitive memory formation with the help of inflammatory mediators. Activation of the HP axis results in the induction of non-catecholamine enzymes like cortisol. The production of cortisol may result in the development of ALB, and SM. The formed ALB and SM showed the microbial stress formed in the oral and gut dysbiosis. Formed stress further results in a higher level of cognitive impairment in the EIGs that receive a higher load of *E. coli* in the form of oral infusions. This infused higher *E. coli* loads results in the conversion of the opportunistic *E. coli* into a virulent one for the formation of cognitive decline, ALB, and SM through the establishment of oral and gut dysbiosis.

Conclusion

The present study has tried to elucidate the role of the opportunistic pathogen (*E. coli*) in forming cognitive memory in their normal and increased state by administering oral microbial infusions in naïve goldfish *C. auratus*. The increased colonization of *E. coli* was achieved in this study with the help of oral microbial infusions in varying ratios. The outcome of the present study came with the initial finding that *E. coli* shiga toxin metabolites may play a major role in the formation of cognitive dysfunction in *C. auratus*. The formed cognitive dysfunction may result from oral/gut dysbiosis due to *E. coli* multiplication. Multiplication of *E. coli* results in the reduction of host beneficial flora in the oral cavity and gut. The reduced/aberrated microflora results in the reduced synthesis of precursor neurochemicals and increased inflammatory mediators. An increase in the inflammatory mediators results in inflammation in the blood-brain barrier, reducing the transportation of precursor neurochemicals and inflammatory mediators to the brain. Transportation of inflammatory mediators

results in neuroinflammation reactions in the hippocampal brain regions. The formation of neuroinflammation may cause hindrances in the memory reconsolidation process in the brain. The impairment in the memory reconsolidation process further results in cognitive dysfunctions in the infused groups compared to the non-infusive group. The outcome of the present study proved that shiga toxin produced by *E. coli* may have a probable role in the development of cognitive dysfunctions through the induction of oral/gut dysbiosis. It also elucidated that oral/gut dysbiosis may also act as a negative regulator of cognitive memory by increasing neuroinflammatory mediators.

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Conflict of interest

The authors declare that they have no conflict of interest.

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