Original Article

# Assessment of locomotion behavior in adult Zebrafish after acute exposure to different pharmacological reference compounds


## Abstract

**Objectives:** The objective of the present study was to assess locomotor behavior of adult zebrafish after acute exposure to different pharmacological reference compounds. **Materials and Methods:** Adult zebrafish of 4-5-months-old were exposed to different concentrations of known reference compounds for 15 min. The test was conducted separately for each drug concentration as well as control. Locomotor activity parameters viz. distance travelled, speed, total mobile time, and total immobile time were recorded for each animal during the exposure period. **Results:** Out of 11 compounds tested, nine compounds showed decrease in locomotor behavior with significant changes in distance travelled, speed, total mobile time, and total immobile time. Caffeine exhibited biphasic response in locomotion behavior, while scopolamine failed to induce any significant changes. **Conclusion:** In view of the above findings, these results suggested that exposure of adult zebrafish with different known compounds produce the expected changes in the locomotion behavior; therefore, adult zebrafish can be used an alternative approach for the assessment of new chemical entities for their effect on locomotor behavior.



**Key words:**
*Caffeine, distance travelled, locomotor activity, scopolamine, speed, zebrafish*


## Introduction

To date, the majority of safety issues concern with central nervous system (CNS) stimulant/depressant activity of new chemical entities (NCEs) has been evaluated using locomotor activity parameter in rodents.[1,2] During drug development, effect on locomotor activity is an undesirable property and is typically detected only in later stage of preclinical safety studies conducted on higher vertebrates.[3] The need for detection of these effects is essential at early stages of drug discovery.

Over the last decade, zebrafish (*Danio rerio*) one of the unique vertebrate models has rose to prominence for assessing drugs *in vivo* with respect to a wide range of toxicological and safety pharmacological evaluation because of fully sequenced genome and highly conserved genetic pathway between zebrafish and human.[4-7] Recent research has revealed the emergence of zebrafish as a model for neurobehavioral studies since zebrafish larvae display learning, sleep, drug addiction, locomotor behavior, and other neurobehavioral phenotype that can be related to those seen in human.[6,8-12] The overall organization of the zebrafish brain is similar to other vertebrates and the blood brain barrier is functional at 10 days post-fertilization (d.p.f.).[13] Zebrafish takes up hydrophilic substance easily from the water through the gills and from entire body surface which makes easy to develop CNS models in zebrafish. In this respect, recent studies identified the


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potential of the larvae zebrafish as well as adult zebrafish for their locomotor behavior.[14-17]

As per our knowledge, very few studies have been performed and published on locomotor behavior of adult zebrafish using drugs with varied therapeutic category. Most of the studies were conducted in zebrafish larvae. From a technical point of view, the small size of larvae makes recording from multiple sites extremely difficult. The mammalian literature suggests that the response to neuroactive drugs may differ as the brain develops and it cannot be assumed that larval zebrafish show the identical response to these drugs as do adult zebrafish.[10]

Therefore, in this study we chose adult zebrafish as an experimental test system in contrast to zebrafish larvae to evaluate the effect of the selected pharmacological reference compounds of various therapeutic categories on locomotor behavior. The reference compounds chosen cover a broad range of therapeutic indications and pharmacological mechanisms and have been subjected to pharmacological validation studies in rodents in the past. Different locomotor activity parameters viz. distance travelled, speed, total mobile time, and total immobile time were recorded using a video tracking system and compared the data of treatment group with the respective vehicle control.

## Materials and Methods

### Animal and maintenance

Around 4-5-month old, stock of adult zebrafish (*Danio rerio*) of either sex was purchased from a local vendor (Big Fish, Gurgaon, India) and acclimatized for at least 1 month before starting the study. For all experiments zebrafish were housed in a 40-L tank, filled with deionized water maintained at 28-30°C and pH = 7.0-8.0 with constant filtration and aeration. Water conditioning and environmental quality was maintained according to the aquarium system use and care manual and The Zebrafish Book.[18] A dark-light cycle of 12-h light:12-h dark (on: 8:00 am; off: 20:00 pm) was maintained. Utmost care was taken to ensure that all the animals were treated humanely. Zebrafish were fed twice daily with tetrabits adult zebrafish diet (Spectrum Brands Company, Germany), and the diet was supplemented with live artemia. Behavioral testing of drug effects took place during the light phase between 10:00 am and 5:00 pm.

### Chemicals and reagents

Caffeine, clonidine, chlorpromazine hydrochloride, scopolamine hydrobromide, and imipramine hydrochloride were purchased from Sigma-Aldrich (Bangalore, India), pentobarbitone sodium was procured from LOBA Chemi (Mumbai, India), diazepam injection (Calmpose), ramelteon, venlafaxine hydrochloride, and rasagiline were procured from Ranbaxy Research Laboratories (Gurgaon, India). All drug solutions were prepared daily in deionized water. Selection of doses was based on the literature report[11] and the preliminary experiments conducted in our laboratory (data not shown). Drugs and their concentrations were as follows: clonidine (100 μM), pentobarbitone (100 μM), diazepam (10 μM), ramelteon (30 μM), chlorpromazine (10 μM), rasagiline (100 μM), imipramine (30 μM), venlafaxine (100 μM), scopolamine (100 μM), and caffeine (1, 100 μM).

### Locomotor activity monitoring

All the zebrafish were divided into 12 groups of 8-10/group/drug concentration except group I which contained 18 fish that served as a control group (deionized water) for all the drugs. The test was conducted separately for each drug concentration. During the test, individual fish was exposed and observed for 15 min for the control group as well as the drug treated group. For analysis of locomotor activity, swimming behavior was monitored using a video tracking system (ANY-maze 4.50, Stoelting Co., USA) and different behavior parameters, for example, distance travelled (m), speed (cm/s), total time mobile (s), and total time immobile (s) were recorded.

### Data analysis

The mean value of distance travelled (m), speed (cm/s), and total time mobile and immobile (s) were calculated from the individual values of each fish for each drug concentration and compared with the vehicle control group (deionized water) using one-way analysis of variance (ANOVA) followed by Dunnett's test. All data are expressed as mean ± standard error of the mean (SEM) for group of size "*n*". $P < 0.05$ and $<0.01$ was considered as the criterion for statistical significance.

## Results

The effect on locomotion behavior of adult zebrafish was evaluated using clonidine (100 μM), pentobarbital (100 μM), diazepam (10 μM), ramelteon (30 μM), chlorpromazine (10 μM), rasagiline (100 μM), imipramine (30 μM), venlafaxine (100 μM), scopolamine (100 μM), and caffeine (1 and 100 μM) [Figure 1]. Different locomotor parameters, for example, distance travelled, speed, total time mobile, and total time immobile of each drug group were compared with the control group.

### Effect on distance travelled and speed

The effect on distance travelled and speed in zebrafish is shown in Figures 2 and 3. Clonidine (48.23 ± 5.71; 5.36 ± 0.63), pentobarbitone (38.25 ± 5.66; 6.49 ± 0.87), diazepam (58.44 ± 7.80; 4.25 ± 0.63), ramelteon (32.94 ± 3.88; 3.65 ± 0.44), chlorpromazine (40.66 ± 5.24; 4.52 ± 0.58), rasagiline (73.76 ± 12.60; 8.20 ± 1.40), imipramine (58.31 ± 10.42; 6.48 ± 1.16), and venlafaxine (30.84 ± 5.38; 3.41 ± 0.60) showed significant ($P < 0.01$) reduction


**Figure 1:** Locomotion tracking plots of individual zebrafish in normal bath medium and in solution of different known marketed test compounds at specified concentration used in this experiment. Each fish was exposed up to 15 min in the bathing medium of control as well as test compounds. Plots were obtained from the video tracking system (ANY-MAZE 4.5)

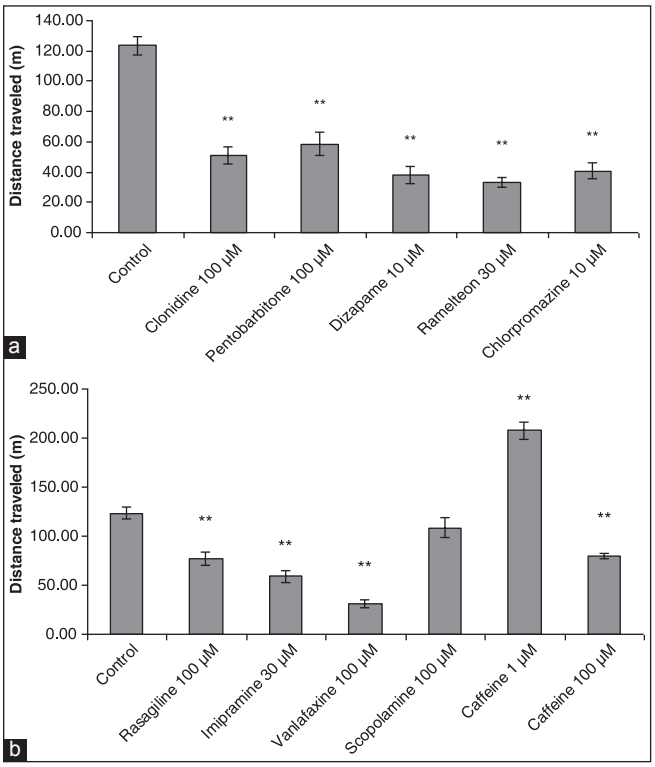


**Figure 2:** (a and b) Effect on distance travelled (m) of zebrafish. Distance travelled are expressed in mean ($n$ = 8-10/group, except $n$ = 18 in vehicle group) ± standard error of the mean (SEM). *$P < 0.05$, **$P < 0.01$ as compared to respective vehicle group (deionized water) (Dunnett's post hoc test following a one-way analysis of variance (ANOVA))

in the distance travelled and speed, respectively, when compared to the control group (123.47 ± 6.23 and 13.71 ± 0.69, respectively) [Figures 2 and 3]. We found that zebrafish exposed to selected reference compounds displayed significant decrease in speed in all the treated groups compared to control group except scopolamine (11.52 ± 0.94) which did not show any significant change in speed. Exposure to caffeine showed biphasic response, that is, at low doses (1μM) a significant ($P < 0.01$) CNS stimulant activity (increase in distance travelled (207.50 ± 8.93) and speed (23.06 ± 1.28)) was observed, while this stimulant effect gradually subsided to depressant effect (decrease in distance travelled (79.54 ± 3.14) and speed (8.84 ± 0.35)) significantly ($P < 0.01$) at higher dose (100 μM) compared to the control group. The results of caffeine produced in this study are similar to the biphasic response reported in rodents.[19]

### Effect on total mobility and immobility time

Effect on total mobility [Figure 4] and immobility [Figure 5] time of each fish was also evaluated for each treatment group and compared with the control group (867.54 ± 17.53 and 38.95 ± 17.53, respectively). The results of reduction in total mobility time and increase in the total immobility time in different groups correlate well with the decrease in speed and distance travelled. These changes were significant in clonidine (552.67 ± 59.02; 347.33 ± 59.02), pentobarbitone (583.50 ± 71.60; 316.50 ± 71.60), ramelteon (693.48 ± 76.23; 206.52 ± 76.23), diazepam (563.99 ± 71.17; 336.00 ± 71.17), venlafaxine (547.13 ± 80.16; 352.87 ± 80.16), and rasagiline (702.14 ± 59.08; 197.86 ± 59.08) group, respectively. Imipramine, caffeine, and scopolamine produced nonsignificant marginal increase in mobility time with decrease in immobility time compared to the control group.

## Discussion

The effect on CNS behavior (convulsion or locomotor activity) in adult as well as larval zebrafish has been extensively studied using narcoleptics[6,8,9] or alcohol.[14,20,21] The present study provides the preliminary evidence that the adult zebrafish may be an excellent tool for early stage pharmacological and/or safety investigation of the new chemical entities for their effects on locomotor behavior and the possible involvement of different receptors and mechanism. To prove this we investigated the effect on locomotor activity of different category of established psychomotor stimulants such as caffeine (inhibitor of adenosine $A_1/A_{2A}$ receptor and phosphodiesterases); scopolamine (centrally

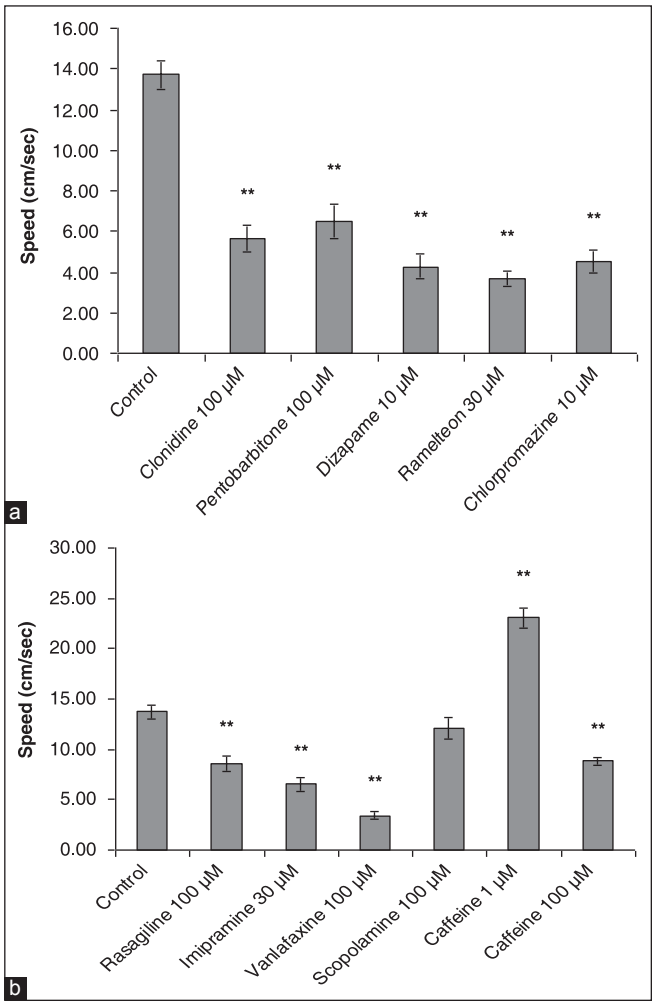


**Figure 3:** (a and b) Effect on speed (cm/s) of zebrafish. Speed are expressed in mean ($n$ = 8-10/group, except $n$ = 18 in vehicle group) ± SEM. *$P < 0.05$, **$P < 0.01$ as compared to respective vehicle group (deionized water) (Dunnett's post hoc test following a one-way ANOVA)

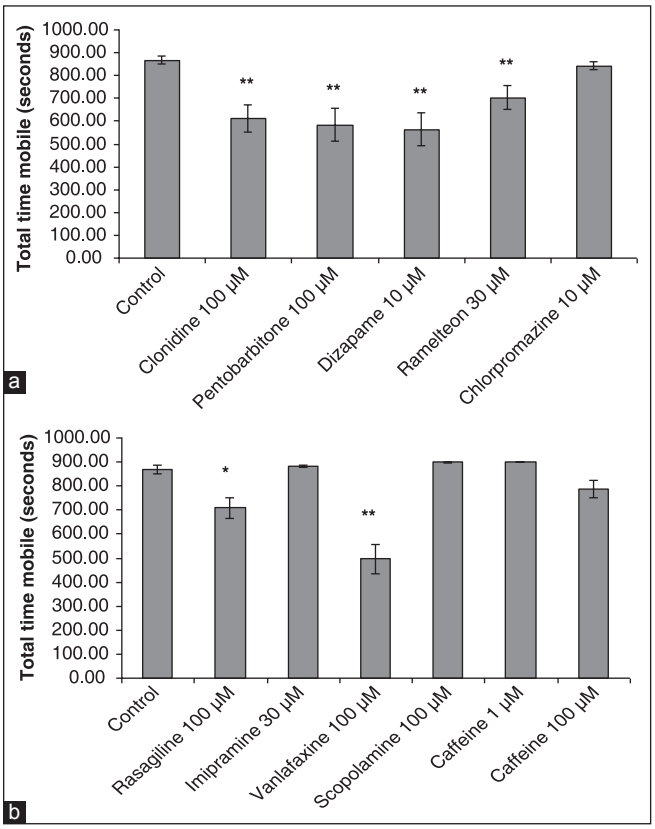


**Figure 4:** (a and b) Effect on total time mobile (s) of zebrafish. Total time mobile are expressed in mean ($n$ = 8-10/group, except $n$ = 18 in vehicle group) ± SEM. *$P < 0.05$, **$P < 0.01$ as compared to respective vehicle group (deionized water) (Dunnett's post hoc test following a one way ANOVA)

acting nonselective muscarinic receptor antagonist with both psychostimulant and depressant properties depending upon dose); and psychomotor depressants, that is, clonidine ($\alpha_2$ agonist), diazepam (benzodiazepine), pentobarbitone (barbiturates), ramelteon ($MT_1/MT_2$ blocker), chlorpromazine (antipsychotic), venlafaxine and imipramine (antidepressant), and rasagiline (anti-Parkinson) on adult zebrafish. As per our knowledge no extensive study has been conducted on zebrafish using these compounds.

To validate this model each fish was exposed into different drug concentrations for 15 min. The reason behind selecting the experiment duration of 15 min is on the basis of study conducted on the effect of alcohol in zebrafish. In this study, brain level of alcohol was estimated after 15 min of exposure and it was found to be 90% of the tank alcohol concentration.[20] By assuming the same achieved concentration with these drugs we exposed each zebrafish up to 15 min. Using zebrafish in the standard procedure, it is observed that out of 11 compounds tested, nine compounds decrease locomotor behavior as anticipated, while caffeine showed biphasic response and scopolamine did not show any changes in locomotor activity.

Clonidine, a known $\alpha_2$ agonist, inhibits noradrenergic activity by acting on presynaptic autoreceptor known to decrease locomotor activity in small animals.[22-24] The findings of our study are in line with above reported studies which support the involvement of noradrenergic pathway in zebrafish.[25] The effect of pentobarbitone, diazepam, and ramelteon (all are known sedatives) on locomotor behavior of zebrafish was evaluated. Benzodiazepines and barbiturates are known to cause sleep promoting effect to the loss of consciousness in humans and other mammals.[11] The inhibitory effect of conventional hypnotic agents (pentobarbitone and diazepam) are due to activation of $GABA_A$ receptors is the major mechanism of depressant/sedative action[26,27] which potentiate the rest behavior in zebrafish as we found in our study. It has

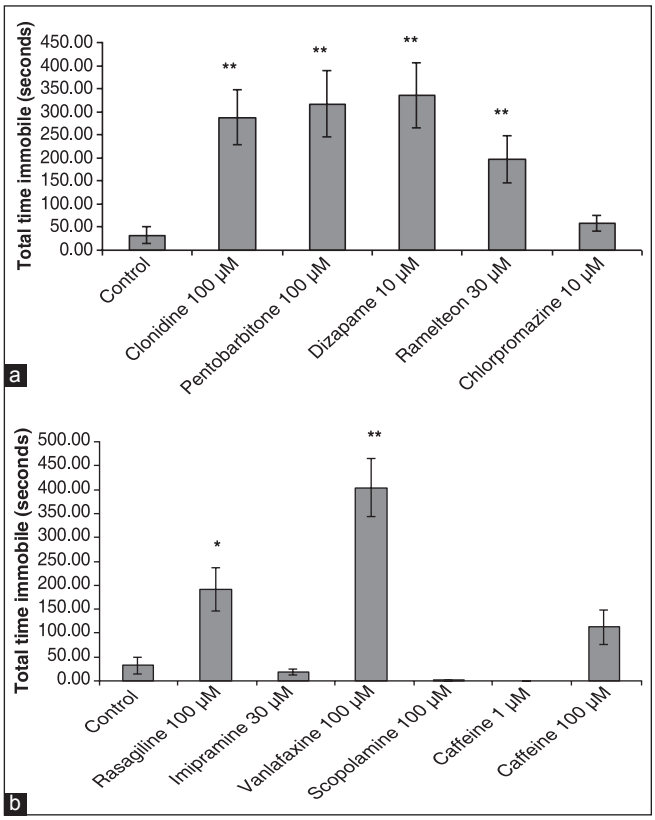


**Figure 5:** (a and b) Effect on total time immobile (s) of zebrafish. Total time immobile are expressed in mean ($n$ = 8-10/group, except $n$ = 18 in vehicle group) ± SEM. *$P < 0.05$, **$P < 0.01$ as compared to respective vehicle group (deionized water) (Dunnett's post hoc test following a one-way ANOVA)

been already reported that the brain of adult zebrafish has higher expression of $MT_1$ receptor than $MT_2$.[28] Ramelteon is a selective melatonin receptor ($MT_1$ and $MT_2$) agonist promotes the effect of melatonin on sleep.[29] Our results of ramelteon in zebrafish showed significant reduction in locomotor activity confirms its action more towards through $MT_1$ receptor. To the best of our knowledge, it's a first kind of study on the effect of locomotion behavior of adult zebrafish using a selective melatonin receptor agonist.

Chlorpromazine a typical antipsychotic drug is known to produce extrapyramidal motor effects associated with blockade of dopamine receptors ($D_2$) in the basal ganglia. Preclinically however, one of the most consistent effects observed with the acute administration of chlorpromazine is suppression of voluntary behavior including motor.[30] Our results are also in agreement with previous findings in rodents showed significant decrease in locomotor behavior supports the involvement of dopaminergic pathway in zebrafish.[31-33]

Rasagiline is reported as a selective irreversible monoamine oxidase (MAO)-B inhibitor at low dose and inhibits MAO-A at higher dose. It has anti-Parkinson and neuroprotective action.[34] Villegier *et al*., observed that concomitant irreversible blockade of both MAO-A and -B, long exposure/chronic treatment, and accumulation of extracellular monoamines may be necessary to induce locomotor activation.[35] Selective inhibition of MAO-B does not cause significant changes in CNS steady state level of norepinephrine (NE), dopamine (DA) and serotonin (5-hydroxytryptamine (5-HT)). However, it has been anticipated that the used dose of rasagiline in this experiment caused unexpected decrease in locomotor activity in zebrafish. High dose of rasagiline may be causing blockade of MAO-A, which is responsible for most of the adverse effects of MAO-A inhibitors[34] or less systemic exposure failed to induce proper response to increase DA and 5-HT level which are required for hyperactivity.

Another class of compounds venlafaxine and imipramine, known antidepressants, has sedation and decreased psychomotor activity as the most common side effects of these drugs. Alterations in monoamine (DA, 5-HT, and NE) level are likely to disturb both psychomotor activity and mood.[36] Previous studies have shown that acute and chronic administration of norepinephrine transporter (NET) inhibitors and serotonin transport protein (SERT) inhibitor alone, decrease motor activity in rodents.[32,36,37] Imipramine is a tricyclic nonselective NE transporter (NET) and serotonin transporter (SERT) inhibitor, while venlafaxine is a selective NET and SERT inhibitor and a moderate inhibitor of DA reuptake that increases the level of NA and serotonin in rat hippocampus[38] and exhibits decrease in locomotor activity of zebrafish after acute exposure up to 15 min as expected.

Among all the tested drugs, scopolamine was the only one which did not show significant effect on locomotion as reported in rodents.[32,39,40] Loss of inhibition of mesopontine cholinergic neurons via muscarinic receptors is one mechanism by which scopolamine could increase locomotion in rodents.[40] In our experiment, nonsignificant increase in total mobility time and decrease in immobility time was observed with no changes in distance traveled and speed of zebrafish compared to control suggesting the probability of low systemic exposure of scopolamine that failed to inactivate muscarinic receptors properly.

In this study exposure of zebrafish to nonselective $A_1$ and $A_{2A}$ receptor antagonist caffeine showed a biphasic response pattern similar to reported in rodents.[19,32,41] Significant decrease in locomotor activity was observed at higher dose which was probably due to $A_1$ receptor blockage and increased locomotor activity found at low doses was probably due to $A_{2A}$ receptor blockage.[19]

Taken together, the data presented here describes that adult zebrafish is sensitive enough to different classes of compounds already known for their effect on locomotor behavior and opens further possibilities to explore the utility of adult zebrafish as a model for initial pharmacological and safety pharmacology evaluation of NCEs.

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