Studies on sensitivity of zebrafish as a model organism for Parkinson’s disease: Comparison with rat model

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ABSTRACT

Objective: To determine the utility of zebra fish as an animal model for Parkinson’s disease (PD) in comparison with rat model. Materials and Methods: MTT assay was performed on rat and zebrafish brain synaptosomal fractions using rotenone as a neurotoxic agent. Quercetin and resveratrol were used as standards to compare anti-apoptotic activity in both organisms. Catalepsy was induced in zebrafish by exposing them to haloperidol (9 µM) solution. Drug-treated groups were exposed to bromocriptine and pramipexole, 30 min prior to haloperidol exposure at the dose of 2, 5, and 10 µg/mL. Swimming speed, time spent in the bottom of the tank, and complete cataleptic time were evaluated to assess behavioral changes. In rats, catalepsy was induced using haloperidol (1.25 mg/kg i.p.). Drug-treated groups received bromocriptine (2.5 mg/kg) and pramipexole (1 mg/kg) orally. Bar test, block test, and locomotor activity were carried out to assess behavioral changes. Results: Resveratrol and quercetin showed comparable inhibition of apoptosis in rats and zebrafish. In anti-cataleptic study, bromocriptine and pramipexole-treated groups showed significant difference (P < 0.05) in behavioral parameters as compared to haloperidol control group in both the experimental organisms. Results obtained from fish model were in correlation with rat model. Conclusion: Findings of the present study revealed that zebrafish model is highly sensitive and can be used for basic screening of drugs against PD.

Key words: Catalpsy, 3-[4,5-dimethylthia zol-2-yl]-2,5-diphenyltetrazolium bromide, zebrafish

INTRODUCTION

Zebrafish (Danio rerio) have been extensively used in genetic and developmental biology studies. Low cost of housing, ease of handling and drug delivery, high mating rate, and gene homology to mammals makes them suitable model organism for human diseases. Unlike other lower model organisms like Caenorhabditis elegans and Drosophila melanogaster zebrafish are vertebrates, hence they resemble more closely to humans.[1]

It is well-established fact that zebrafish also exhibits various neurobiological responses. Like other vertebrates, zebrafish exhibit cognitive, sensorimotor, and basic motor responses which are controlled by central nervous system. These features make them best candidates for neurochemical and behavioral studies.[2]

Parkinson’s disease (PD) is the second-most common neurodegenerative disorder after Alzheimer’s disease. It is

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known to affect nearly 1% of world population above 60 years of age. It is characterized by progressive loss of dopaminergic neurons in substantia niagra and pars compacta; which on later stages extends to other parts of brain including raphe nuclei (serotonergic neurons), locus coeruleus (noradrenergic neurons), and also to cortex. [3]

Some of the toxins like rotenone, paraquat, MPTP which are known to produce PD like symptoms in mammals, also cause dopaminergic loss in zebrafish. Anti-psychotics like haloperidol which act by temporary blockade of dopaminergic neurons are known to produce cataleptic movements in zebrafish leading to aberrant swimming patterns (upside down, circular, arrow like swimming toward bottom).[4,5]

Further, various proteins like parkin (shares 62% homology with human genes), LRRK2, DJ-1 (83% homologous to human counterpart) Pink1, Trap1, α-synuclein, Ubiquitin C-terminal hydrolase L1 (70% homologous to human genes); playing crucial role in pathology of PD are expressed in zebrafish.[6,4] Thus, considering the above facts, sensitivity of zebrafish model was evaluated by comparing it with rat model using MTT assay against rotenone and haloperidol-induced catalepsy.

In the present study, in vitro MTT assay was performed in synaptosomal fractions of rat and zebrafish brain by rotenone-induced apoptosis. Resveratrol and quercetin were used as standards for comparison.

In addition, we studied the anti-cataleptic effect of bromocriptine and pramipexole in zebrafish, which was then compared with their effect on Wistar rats to determine the utility of zebrafish as an animal model for PD.

**MATERIALS AND METHODS**

**Animals**

Male Wistar rats procured from Haffkine institute, Mumbai were used for the study. They were acclimatized in the animal house of Bombay College of Pharmacy (BCP). Animals were fed standard diet and water was given ad libitum. Twelve hours light/dark cycle was maintained. All animal protocols were approved by IAEC of BCP.

Zebrafish were procured from Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai. Adult wild-type AB strains of zebrafish (3-5 cm) of both the sexes (4-6-months old) were used. The fishes were habituated to the laboratory conditions for at least 14 days and housed in a 50-L tank filled with un-chlorinated aquarium water at temperature of 28 ± 2°C with constant filtration and aeration. Density of five fishes per liter was maintained. Animals were kept on 14:10 h light/dark cycle and were fed twice a day with aquarium food supplemented with brine shrimp eggs.[9]

**Drugs and chemicals**

Bromocriptine (Inga Pharma Pvt. Ltd., Mumbai) and Pramipexole (Sun Pharma, Mumbai) were obtained as gift samples. Haloperidol (Inj. Serenate) RPG Life Sciences, India. MTT and Rotenone were procured from Sigma Aldrich, India.

**In vitro MTT assay**

In the present study, in vitro MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed on synaptosomal fraction of zebrafish and rat brain using rotenone as neurotoxic agent.

**Isolation of synaptosomal fractions**

Three rats were sacrificed by cervical dislocation and their brains were immediately removed and homogenized in sucrose (0.32 M). Homogenates were centrifuged at 3000 rpm for 10 min, and supernatants were then re-centrifuged at 8000 rpm for 15 min. The resultant pellets were resuspended in 40 mL HEPES buffer (pH 7.4). Synaptosomal fractions were then preserved at –70°C until the experiments were performed.[10,11]

Zebrafish were sacrificed by putting them in freezing water, and then brains of 50 fishes were isolated and pooled in order to get adequate volume of fraction. Synaptosomal fraction from fish brain was isolated by performing similar steps as followed for rat brain.[10]

**Estimation of mitochondrial function**

Aliquots of 400 µL containing synaptosomal fractions were incubated in the presence of rotenone (1 mM) and/or quercetin, resveratrol (5 and 10 µg/mL) at 28 ± 2°C (zebrafish) and 37 ± 2°C (rats) for 120 min. Eight microliters of MTT (5 mg/mL) was then added and mixtures were incubated at 37°C for 12 h. Quantification of formazan was made by estimation of optical density at a wavelength of 570 nm on Jasco V-530 UV visible spectrophotometer. Results were expressed as the percentage of MTT reduction (% viability) with respect to control values.[11] Mean absorbance of control group was considered as 100% viability. Percent viability of other treatment groups was calculated with respect to control group.

**In vivo behavioral studies**

**Haloperidol-induced catalepsy in rats**

Rats were randomly divided into four groups (n = 6), viz., vehicle control (vehicle treated), haloperidol control (only Haloperidol treated), bromocriptine-, and pramipexole-treated group.

Bromocriptine and pramipexole were administered orally as a suspension in sodium carboxy methyl cellulose (0.5%) at a dose of 2.5 and 1 mg/kg, respectively. One hour after the drug administration, the animals were challenged with haloperidol 1.25 mg/kg intraperitoneal administration and catalepsy was
evaluated using standard bar test and block test at 0 (before haloperidol administration), 30-, 60-, 120-, 180-, and 240-min time interval.[12] Locomotor activity was evaluated using actophotometer.

**Standard bar test**

The rat forepaws were placed on a 12-cm-high horizontal bar located in a sound attenuated area with background white (static) noise.

Catalepsy was measured for 3 min and each animal underwent three consecutive trials with 5-10 min interval between the tests. An animal was considered cataleptic if both forepaws remained on the bar for at least 1 min. Catalepsy score (immobility time in seconds) of each animal was analyzed by calculating mean scores.[12]

**Block test**

Catalepsy was evaluated using block test by scoring method as follows:

- Step I: The rat was taken out of the home cage and placed on a table. If the rat failed to move when touched or pushed gently on the back a score of 0.5 was assigned
- Step II: The front paws of the rats were placed alternately on a 3-cm-high block. If the rat failed to correct the posture within 15 s, a score of 0.5 for each paw was added to the score of step I
- Step III: The front paws of the rat were placed alternately on a 9-cm-high block, if the rat failed to correct the posture within 15 s a score of 1 for each paw was added to the scores of steps I and II. Thus, the highest score for any animal was 3.5 (cut-off score) and that gives total catalepsy.[12]

**Test for locomotion using actophotometer**

Locomotor activity was evaluated using actophotometer. Ambulation was expressed in terms of total photo beam counts/5 min/animal.[13]

**Catalepsy in Zebrafish**

Fish were divided into eight groups ($n = 5$), viz., vehicle control (vehicle treated without challenge), haloperidol control (haloperidol with vehicle), bromocriptine- (2, 5, and 10 µg/mL), and pramipexole treated group (2, 5, and 10 µg/mL). Behavioral testing was done during day phase, i.e., between 10:00 am and 5:00 pm.

Each fish from bromocriptine- and pramipexole-treated groups were individually exposed to the solution of respective drugs at the concentrations of 2, 5, and 10 µg/mL in a 300-mL beaker for 30 min. Once this exposure was given, then fish were transferred to another beaker containing fresh tank water, where they were kept for 15 min, then fish from all treatment groups were individually transferred to fresh 300-mL beaker containing 9-µg haloperidol solution, where they were kept for another 30 min. After haloperidol exposure, fish were shifted to the examination tank to evaluate various cataleptic parameters, where they were habituated for 5 min. Examination tank [Figure 1] was filled with fresh aerated tank water. It consisted of a 5-L tank ($30 \times 15 \times 10$ cm, length × height × width)[13] with number of vertical lines drawn on one of the faces of tank at the spacing of 5 cm and with one horizontal line which divides the water filled portion of the tank into two equal halves. These vertical lines were used to calculate the speed of fish by measuring the time taken by fish to travel from first vertical line to last and horizontal line gave idea about the time spend in the upper and lower half of the tank by the fish. All behavioral evaluations were done using a web camera (Microsoft life cam). Behavioral study was done for 1 h and parameters were measured at 15-min time interval, viz., 0, 15, 30, 45, and 60. Since after 1 h all fishes recovered from the effect of haloperidol, hence, examination time was standardized to 1 h. Recording was done for 5 min at every time interval and average readings during 5 min were calculated for every individual fish at each time point.

Following behavioral parameters in zebrafish were evaluated:

- Latency to travel from one fixed point to another:
  In this time taken by the fish to travel from first vertical line to last was calculated. This gives an idea about the speed of fish under examination.
- Complete cataleptic time:
  Time for which the fish did not move at all, i.e., the time for which fish remained completely cataleptic during 1 h examination period.
- Time spent near the bottom of the tank:
  Time spent below the horizontal line drawn on the examination tank was measured at different time intervals. This gave an idea about the anxious behavior of the fish under study.

In addition to this, visual observations were made throughout the experiments and any erratic swimming pattern like vertical swimming, sideway swimming, upside-down, arrow-like swimming (darting behavior) were noted down.[4]

**Statistical analysis**
Graph-pad prism 5 was used for all statistical calculations. Data are expressed as mean ± SEM. One-way ANOVA was applied using Dunnet’s test as post hoc test. A significant difference was attributed to value less than \( P < 0.05 \).

RESULTS

In MTT assay, it is evident from Figure 2 that quercetin at 5 and 10 \( \mu \text{g/mL} \) exhibited 65.25% and 88.40% viability, respectively, while resveratrol showed 71.28% and 94.20% viability at 5 and 10 \( \mu \text{g/mL} \), respectively. Comparable results were obtained in zebrafish, viz., quercetin 5 and 10 \( \mu \text{g/mL} \) exhibited 50.99% and 74.42%, respectively, resveratrol 5 and 10 \( \mu \text{g/mL} \) showed 55.29% and 85.87% viability, respectively.

Haloperidol-induced catalepsy in rats

In bar and block tests [Tables 1 and 2], haloperidol control group was found to be significantly different from vehicle control, i.e., the group of normal animals \( (P < 0.05) \) at all the time intervals evaluated. Bromocriptine and pramipexole showed significant \( (P < 0.05) \) inhibition against catalepsy in both tests. Although there was no significant difference between both the drug treatment groups.

Locomotor activity

Reduction in locomotion induced by haloperidol was successfully regained by bromocriptine and pramipexole to normal [Table 3].

Catalepsy in Zebrafish

Latency to travel from one point to another

Haloperidol significantly decreased the latency to travel from one fixed point to another and increased the complete cataleptic time and time spent near the bottom of the tank as compared to vehicle control group. It can be observed from Table 4 that groups treated with bromocriptine 5 \( \mu \text{g/mL} \) and 10 \( \mu \text{g/mL} \), pramipexole 10 \( \mu \text{g/mL} \) significantly recovered latency to travel from one point to another; as compared to haloperidol control group.

Complete cataleptic time

Haloperidol control significantly increased the complete cataleptic time in zebrafish. Bromocriptine and pramipexole 2, 5, and 10 \( \mu \text{g/mL} \) produced drastic and statistically significant reduction in complete cataleptic time as compared to haloperidol control group [Table 5].

Time spent near the bottom of the tank

This parameter gives the index of anxiety in fishes. Haloperidol control group was significantly different from vehicle control. Bromocriptine 5 and 10 \( \mu \text{g/mL} \) and pramipexole 10 \( \mu \text{g/mL} \) exhibited significant reversal of the anxious behavior at 45 and 60 min as compared to haloperidol control group [Table 6].

DISCUSSIONS

In vitro MTT assay

The MTT reduction assay is an in vitro assay for the measurement of cell viability. Several metabolic events can lead to apoptosis or necrosis leading to a reduction in cell viability. MTT is a yellow tetrazolium dye, which gets reduced to violet color product called formazan in the presence of active mitochondrial

Table 1: Effect of bromocriptine and pramipexole on catalepsy in bar test

| Time interval in mins | Vehicle control | Haloperidol control 1.25 mg/kg i.p. | Bromocriptine 2.5 mg/kg p.o. | Pramipexole 1 mg/kg p.o. |
|----------------------|----------------|-------------------------------------|-----------------------------|------------------------|
| 0                    | 1.17±0.17      | 1.64±0.50                           | 1.35±0.32                   | 1.50±0.30              |
| 30                   | 1.37±0.52      | 16.05±1.39*                         | 2.50±0.30**                 | 2.03±0.40**            |
| 60                   | 0.77±0.26      | 76.47±17.36*                        | 2.40±0.32**                 | 1.67±0.15**            |
| 120                  | 1.52±0.66      | 148.08±11.16*                       | 3.52±0.41**                 | 2.24±0.53**            |
| 180                  | 2.89±1.06      | 166.90±11.49*                       | 4.0±0.48**                  | 2.75±0.41**            |
| 240                  | 1.93±0.27      | 168.00±10.33*                       | 8.78±0.67**                 | 3.06±0.40**            |

All values are expressed in Mean±SEM of catalepsy in secs. \((n=6)\). *Haloperidol control group was found to be significantly \((P<0.05)\) different as compared to vehicle control group. ** Treatment groups were found to increase ambulation counts significantly \((P<0.05)\) as compared to haloperidol control group.
Table 2: Effect of bromocriptine and pramipexole on catalepsy in block test

| Time interval in mins | Vehicle control | Haloperidol control | Bromocriptine 2.5 mg/kg p.o. | Pramipexole 1 mg/kg p.o. |
|-----------------------|----------------|---------------------|-----------------------------|--------------------------|
| 0                     | 0.00±0.00      | 0.33±0.16           | 0.0±0.0                     | 0.0±0.0                  |
| 30                    | 0.00±0.00      | 1.50±0.22*          | 0.16±0.10**                 | 0.25±0.11**              |
| 60                    | 0.00±0.00      | 3.16±0.16*          | 0.83±0.21**                 | 0.58±0.15**              |
| 120                   | 0.00±0.00      | 3.41±0.08*          | 1.50±0.22**                 | 0.75±0.17**              |
| 180                   | 0.00±0.00      | 3.41±0.08*          | 1.50±0.12**                 | 0.91±0.20**              |
| 240                   | 0.00±0.00      | 3.41±0.08*          | 1.58±0.20**                 | 1.75±0.30**              |

All values are expressed in Mean±SEM of cataleptic score (n=6). *Haloperidol control group was found to be significantly (P<0.05) different as compared to vehicle control group. ** Treatment groups were found to increase ambulation counts significantly (P<0.05) as compared to haloperidol control group.

Table 3: Effect of bromocriptine and pramipexole on locomotor activity using actophotometer in rats

| Treatment groups | Ambulation counts/5 min (Mean±SEM) |
|------------------|-----------------------------------|
| Vehicle control  | 453.25±33.07                      |
| Haloperidol control | 89.95±25.08*                    |
| Bromocriptine 2.5 mg/kg | 421.99±29.07**               |
| Pramipexole 1 mg/kg  | 409.19±10.61**                  |

All values are expressed in Mean±SEM of ambulation counts (n=6). *Haloperidol control group was found to be significantly (P<0.05) different as compared to vehicle control group. ** Treatment groups were found to increase ambulation counts significantly (P<0.05) as compared to haloperidol control group.

reductase, which are present in the viable cells. The rate of tetrazolium reduction is proportional to cell viability.[14]

In the present study, MTT assay was done on zebrafish and rat synaptosomal fraction of brain using standard apoptotic inhibitors like quercetin and resveratrol, in order to compare the efficiency of rat and zebrafish model. Rotenone was used as neurotoxic agent against whom the protective effect of drugs was evaluated. Rotenone is a potent specific inhibitor of mitochondrial complex-I also known as NADH: Ubiquinone oxidoreductase leading to depolymerization of microtubules causing rupture of transport vesicles. Hence, rotenone causes significant inhibition of MTT reduction.[15]

From results, it is clear that the protective effect of quercetin and resveratrol in viability was found to be comparable in rats and zebrafish fractions.

Thus, in vitro MTT assay can be easily used on brain synaptosomal fraction of zebrafish to study neuroprotective effects of drugs as it produced comparable results with that of rat.

Haloperidol-induced catalepsy in rats
Catalepsy (rigidity in movements), akinesia (slowing of movement), tremors, and loss of memory are some of the major symptoms of PD. Amongst this rigidity, i.e., catalepsy is one of the major symptoms which make life of PD patient uncomfortable.[16] Bromocriptine and pramipexole are well-known dopamine (D2) receptor agonists and are commonly used to improve the symptoms related to rigidity.[17,18] Hence both the drugs were used as standards in present study to compare the efficiency of both the models (rat and zebrafish).

Catalepsy was induced in rats by intraperitoneal administration of haloperidol (1.25 mg/kg). This cataleptic behavior induced by haloperidol and protective effect of standards used was evaluated by using bar and block test. Both the test gives idea about the extent of catalepsy induced in an animal.[12]

In the present study, bromocriptine and pramipexole reversed the effects of haloperidol in bar and block test.

Locomotor activity
Due to catalepsy or rigidity; restricted movements or sometimes freezing of movements are exhibited by PD patient. Hence, drug improving the locomotor activity can modify the condition of PD patient.[16]

As bromocriptine and pramipexole are D2 agonist, they exhibited promising potential against restricted locomotion induced by haloperidol which is in agreement with the previous reports.[17,18]

Catalepsy in zebrafish
Catalepsy was induced in zebrafish using standardized dose of haloperidol (9 μg) by giving direct exposure to fishes. It was observed that the effect of haloperidol lasts for an hour in a fish,[9] hence study period of 1 h after haloperidol exposure was standardized and kept constant. Bromocriptine and pramipaxole were used to compare the efficiency of fish model with respect to rat model. These drugs being insoluble in water were first solubilized in 0.5% DMSO containing water (well below the permitted limit of DMSO in fish) and given as exposure to the fish. Both the drugs were tried arbitrarily at different concentrations, viz., 2, 5, 10, and 20 μg/mL but ceiling effect after 10 μg/mL in case of both the drugs was observed hence, doses...
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Catalepsy was successfully induced in fish by haloperidol. During induction of catalepsy, fish started showing aberrant swimming patterns like upside down, arrow like swimming, circular swimming, and finally state of complete catalepsy was achieved [Figure 3], which is in agreement with previously reported studies.\[9\]

Various behavioral parameters studied in fish were

- Latency to travel from one point to another:

Catalepsy diminishes the speed of fishes due to rigidity of muscular movements.\[10-20\] In the present study, after induction of catalepsy, rigidity of fins was observed due to which there was a difficulty in swimming experienced by the fishes. Thus, they took longer to travel from one particular point of tank to another. Keeping this into consideration, time taken by fish to travel from first vertical line of examination tank to last line was measured at every time interval during the study period.

  - Complete cataleptic time:

Time for which the fish were in completely immovable state was used as index of locomotor activity in fishes.\[2\] Figure 3c indicates the state of complete catalepsy when exposed to haloperidol solution. This parameter was studied to evaluate the efficiency of zebrafish model by studying the protective effect of bromocriptine and pramipexole.

  - Time spent near the bottom of the tank:

It is a well-known fact that zebrafish are surface fish, i.e., they swim near the surface of the water. When they are transferred to a new environment (tank) they initially spend more time near the bottom of the tank and after sometime they come toward surface, this is attributed towards their exploratory and most often due to their anxiety. Thus, the time spent near the bottom of the tank gives idea about the extent of anxiety of fish.\[2,23,24\] Here, it was calculated by measuring the time spent by the fish below the horizontal line drawn on the examination tank.

Haloperidol model in rats is well established and very commonly used.\[12,13,25\] Zebrafish have tendency to swim from one side of the tank to another and near the surface of water.\[2,23\] But these behaviors were completely abolished by haloperidol. Fishes started swimming erratically, i.e., in upside down, arrow-like, or in circles and spending more time near the bottom of the tank. All these erratic behaviors were reversed by bromocriptine and pramipexole. Diminished swimming speed due to haloperidol was also recovered by bromocriptine and pramipexole. This suggests that dopaminergic system of zebrafish works like mammalian dopaminergic system. Hence it can be perfect model organism for PD.

Besides this, our study revealed number of advantages in using

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**Table 4: Effect of bromocriptine and pramipexole on latency to travel from one fixed point to another (Sec)**

| Treatment groups | Latency to travel from one particular point to another in Sec Mean±SEM |
|------------------|-----------------------------------------------------------------------|
|                  | 15                        | 30                        | 45                        | 60                        |
| Negative control | 11.03±0.49                | 11.07±0.33                | 10.02±0.90                | 10.47±0.46                |
| Positive control | 24.87±2.00*               | 22.66±0.67*               | 19.78±0.54*               | 16.15±1.10*               |
| Bromocriptine 2 μg/mL | 22.84±0.60               | 19.38±0.69                | 16.98±0.91                | 10.60±0.17**              |
| Pramipexole 2 μg/mL | 25.81±2.29                | 23.00±1.67                | 18.46±0.67                | 13.45±1.67                |
| Bromocriptine 5 μg/mL | 15.71±0.78**              | 12.28±0.30**              | 11.36±0.21**              | 9.72±0.27**               |
| Pramipexole 5 μg/mL | 21.86±2.87                | 19.38±2.22                | 10.10±0.57**              | 14.20±1.96                |
| Bromocriptine 10 μg/mL | 14.95±0.38**              | 11.60±0.39**              | 10.05±0.75**              | 10.17±1.06                |
| Pramipexole 10 μg/mL | 15.79±1.19**              | 11.77±1.19**              | 12.53±0.84**              | 9.22±1.08**               |

All values are expressed in Mean±SEM seconds (n=5). *Haloperidol control group significantly (P<0.05) increased the time spent near the bottom of the tank as compared to vehicle control group. **Treatment groups were found to significantly (P<0.05) decrease the time spent near the bottom of the tank as compared to haloperidol control group.

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**Table 5: Effect of bromocriptine and pramipexole on complete cataleptic time (Sec)**

| Treatment groups | Complete cataleptic time in secs (Mean±SEM) |
|------------------|--------------------------------------------|
| Vehicle control  | 0.00±0.00                                  |
| Haloperidol control | 278.66±38.10*                            |
| Bromocriptine 2 μg/mL | 7.00±2.08**                             |
| Bromocriptine 5 μg/mL | 1.11±0.57**                             |
| Bromocriptine 10 μg/mL | 0.4±0.07***                             |
| Pramipexole 2 μg/mL | 28.00±4.35**                             |
| Pramipexole 5 μg/mL | 13.32±3.18**                             |
| Pramipexole 10 μg/mL | 3.95±1.00**                              |

All values are expressed in Mean±SEM seconds (n=5). *Haloperidol control group significantly (P<0.05) increased the time spent near the bottom of the tank as compared to vehicle control group. **Treatment groups were found to significantly (P<0.05) decrease the time spent near the bottom of the tank as compared to haloperidol control group.
Table 6: Effect of bromocriptine and pramipexole on time spent near the bottom of the tank (Sec)

| Treatment groups          | Time spent in bottom of the tank in Sec Mean±SEM |
|---------------------------|-----------------------------------------------|
|                           | 15                            | 30                       | 45                        | 60                        |
| Vehicle control           | 29.66±7.68                    | 24.66±10.25              | 11.29±0.35                | 24.00±16.37               |
| Haloperidol control       | 214.66±13.37*                | 164±20.66*               | 195±22.72*               | 15±19.28*                |
| Bromocriptine 2 μg/mL     | 203.33±15.34                  | 221.66±5.20              | 147.66±16.50             | 146.00±16.09             |
| Pramipexole 2 μg/mL       | 224.66±21.72                 | 212.33±29.42             | 203.33±15.34             | 221.66±5.20              |
| Bromocriptine 5 μg/mL     | 173.00±8.62**                | 208.33±15.37             | 107.00±9.45**            | 49.00±14.18**            |
| Pramipexole 5 μg/mL       | 203.66±15.07                 | 154.66±16.90             | 150.66±16.75             | 95.00±12.85**            |
| Bromocriptine 10 μg/mL    | 170.66±21.16**               | 159.00±19.75             | 140.00±9.53**            | 43.33±8.68**             |
| Pramipexole 10 μg/mL      | 260±6.96                     | 203.66±15.07             | 195.00±12.85             | 147.66±16.50             |

All values are expressed in Mean±SEM in sec (n=5). *Haloperidol control group significantly (P<0.05) increased the time spent near the bottom of the tank as compared to vehicle control group. **Treatment groups were found to significantly (P<0.05) decrease the time spent near the bottom of the tank as compared to haloperidol control group.

Figure 3: Induction of Catalepsy in zebrafish in haloperidol solution: (a) Fish just introduced in haloperidol solution (b) Aberrant swimming patterns shown by fish after some time (c) State of complete catalepsy

zebrafish as a model organism. Time required in zebrafish study was very less as compared to rat (1 h for fish and 4 h for rat). Reproducibility was similar in both the models. Quantity of drugs required in zebrafish was very less in micro-molar level as compared to milligrams required in rats. Cost effective with very less husbandry and maintenance cost as compared to rodent facility.

CONCLUSIONS

In conclusion, we can say that zebrafish may become effective tool for high throughput screening for various diseases. They can be used with ease and effectiveness for initial screening of drugs before subjecting them to rodent testing. Thus saving number of rodents and also it satisfies 3R's of pharmacological testing.

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