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Research Article

EFFECTS OF ANTI ANXIETY DRUG MIDAZOLAM AS PRETREATMENT THERAPY FOR ANXIETY OF ZEBRA FISHES INDUCED BY THE EXPOSURE OF UNFAMILIAR ENVIRONMENT OF AQUATIC WHITE/BLACK MAZE

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ABSTRACT

Objectves: The objective of our present research investigation was to evaluate the effectiveness of the aquatic white/black plus maze model to evaluate the efficacy of different treatment drugs on zebra fish behavior studies.

Methods: Animals were divided into three groups each containing six animals. The first two groups were pretreated with lower and higher doses of midazolam and then they were exposed to unfamiliar environments of aquatic white/black plus maze. The last group of animals was only immersed into the unknown familiar in the form of aquatic white/black plus maze without any pretreatment of the drugs.

Results: The results illustrated that the pretreatment drugs certainly improved the behavior status of the animal in the form of releasing anxiety and stress, but the doses of different pretreatment have no significant effects on improvement of behavioral status.

Conclusion: In conclusion, it can be described that this model is highly effective to find the proper drug for the treatment of anxiety but it is not so effective to find the proper dose of treatment.

Keywords: Midazolam, Zebra fish, White/black maze, Anti anxiety, Pretreatment therapy.

INTRODUCTION

The zebra fish was recently used as an animal species to investigate different kind of neurobehavioral studies of different drugs. The main advantage of these fishes is that they are small, easy to control, cheap, and they have a lot of similarities in complex neurobehavioral studies as compared to human beings [1-4]. After thorough literature survey, it was found that zebrafish was used as experimental species to find a different model for investigation of neurobehavioral research studies. Such models provide a clear insight into neurobehavioral symptoms of different kind of fear and anxiety [5-7]. The most popular among these models are predator model, novel dive tank model, etc [8]. Here, we have investigated an aquatic white/black plus maze to find the initial response to unfamiliar environments condition [9-18]. This model although was vigorously investigated but the effect of pretreatment drug with anxiety drug like midazolam was not investigated thoroughly [19-21]. So here, we attempted to perform an experiment on the basis of the effect of pretreatment with various doses of midazolam on zebrafish when it was immersed into the novel aquatic white/black plus maze. Midazolam is an anti-anxiety drug which is enormously used for the treatment of different kind of anxiety disorder in human beings. In our research studies, we wanted to explore the rationality and efficacy of midazolam at different dose of treatment to cure the anxiety of zebra fishes induced by aquatic white/black plus maze model. The whole experimental study could enhance our understanding about the treatment of behavioral anxiety when it was originated due to exposure of an unfamiliar environment. We have investigated different behavior parameters to evaluate the exact neurobehavioral symptoms of the animals when it was in a stressed condition.

In conclusion, it can be described that this new model will help us to find the effective treatment for different kind of neurobiological disease caused by the unknown and unfamiliar environment imposed on zebra fishes. The result can easily translate to neurobehavioral studies of human beings as the zebra fishes possess lots of neurobiological similarity as that of human beings. Overall, it could be postulated that the research investigation will help the scientific community to deliver a novel anti-anxiety drug for the treatment of different kinds of fear and stress which is induced due to unfamiliar situation and atmosphere.

METHODS

Animals

Adult zebrafish of breeds were procured from a local commercial supplier (Blu Aquarium, Jayanagar and Prabu Aquarium, Pipeline Road, India). The fishes were identified and authenticated by Department of Zoology, Bangalore University. All the animals were kept in water tank (60 cm Length × 40 cm Height × 35 cm Weight) for 30 days period of acclimatization. Before starting of the investigation, all the experimental tanks including acclimatization tank were cleaned by 132 µl/l Aqua Safe solution. The experimental tanks were filled with non-chlorinated water and maintained under mechanical and chemical filtration at a temperature of 26±2°C at pH 7.4. The room illumination was provided by ceiling-mounted lamp according to light/dark cycle (light on at 7:00 am and light off at 7:00 pm). Commercial grade food (Aini Fish Food, Taiyo Pet Products Pvt. Ltd., Kolathur, Chennai) was provided twice a day for all the animals kept in the water tank. The experimental animals were in healthy condition with an average weight 35±2 mg without any visual abnormality. The experiment was conducted according to the National Institute of Health Guide for Care and Use of Laboratory Animals (2011).

Reagents

Midazolam drug was procured from (Ranbaxy Laboratories Limited, Solan, Paonta Sahib, Himachal Pradesh).

Experimental design

The animals were immersed into the main tank where it was acclimatized for a minimum period of 3 weeks before starting the experiment.

Individual fish was netted from the fish tank and was immersed into the center of the section of the white/black plus maze for 5 minutes. All the animals for this experiment were divided into three groups each containing six animals. The two groups of animals were pretreated with higher and lower doses for 5 minutes and then they were immersed into the aquatic white/black plus maze for 5 minutes. The last group which was considered as the control group was directly immersed into the novel aquatic white/black plus maze without pretreatment of the drug midazolam. The responsive behavior was observed with a digitally record camera. During this experiment time spent in center, time spent in white zone, number of entry to white zone, time spent in black zone, and number of entry into black zone were plotted against the time of experiment. The different behavioral parameters that were discussed before was observed and recorded during the time of the experiment conducted for 5 minutes. The whole study is represented in schematic Diagram 1.

Dose selection of midazolam dose

To find the effective midazolam dose for induction of anti–anxiety, an acute toxicity study in the form of LC50 calculation was done on zebra fishes by probit analysis as per OECD guideline [22,23]. The experimental animals were immersed into different concentration of drugs for 96 h to find the mortality rate. 50% animals of the total population were found to be died in the concentration of 5 mg/l midazolam solution which was considered as LC 50 value of zebra fishes. The pretreatment dose was selected as 250 µg/l and 500 µg/l that were 20 and 10 times lesser than LC50 value.

Statistical analysis

Non-parametric data of seizure score data were expressed in terms of mean ± standard error (SE). Cumulative frequency was determined as the percentage of zebra fishes that reached each individual score across different time interval for different treatment used. The area under the curve in terms of score intensity, latency period, and wash out period was represented as mean ± SE followed by one-way ANOVA test and *post-hoc* test. Student's t-test was used to compare different parameters for different doses including pretreatment dose. The caffeine quantification in brain was expressed as mean ± SE. In all the cases, 95% confidence interval was used for finding statistical significance.

RESULTS

Fig. 1 illustrated the time spent in the center of aquatic white/black plus maze. If the animal was in stressed, then time spent in the center was lesser. Here, we found that pretreatment with midazolam in higher and lower dose of the treatment group the animals effectively than as compared to pretreatment control group. The test results also indicated that time spent in the center in high and lower dose was not statistically different ($p \geq 0.05$). The time spent in the center not changed vigorously during the time of the experiment for all the treatment case. This also indicated that as the time elapsed stress remained a little bit constant throughout the experimental studies.

Fig. 2 represents the time spent in the white zone during the time of the experiment for the different dose of treatment. Here, the test with low and high dose displayed a statistically significant difference than as compare to control group. But the difference between test with low and high dose did not show a significant difference ($p \geq 0.05$). The results also illustrated that the time spent in the white zone showing a Plato phase during the time of the experiment for the 5 minutes.

Fig. 3 illustrates the number of entry into the white zone from the dark zone. If the animals were in stressed, then the numbers of entry of the animals into the white zone from the dark zone decreased significantly. Here, we found that the control group of the animals was in the high stressed as the number of entry into the white zone significantly quite lesser than as compare to test with higher and lower doses. But the

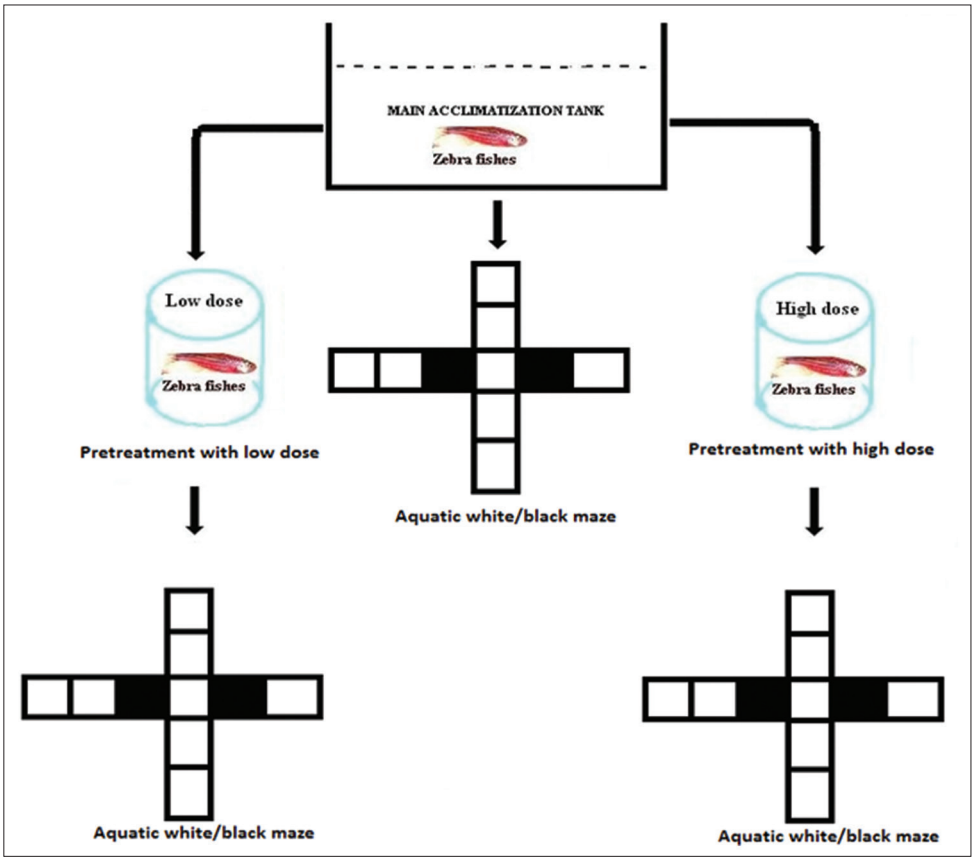

Diagram 1: Schematic diagram of entire experimental studies of aquatic white/black maze including different dose of pretreatment groups

difference between test with lower and higher doses was not found any significant difference (p≥0.05).

Fig. 4 represents the time spent in the black zone at different time interval during the entire experiment. Stressed and anxiety induced animals generally spent more time in the black zone than that of normal and stress-free animals. Here, the results clearly indicated that time spent by the zebrafish in the black zone area are quite lesser than as compare to low and high test group of midazolam. But the test group with lower and higher dose did not produce any significant difference (p≤0.05).

Fig. 5 is plotted as the number of entry into black zone at different time interval. The results clearly indicated when the animals were in stressed, number of entry to the black zone increased vigorously. Here, the control groups of animals have more number of entries into the black zone as compared to lower and higher dose (p≤0.05). The number of entries with higher doses and lower doses did not show any significant difference (p≥0.05).

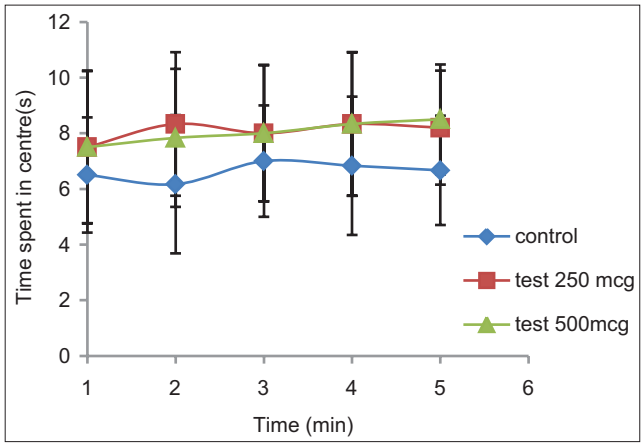

Fig. 1: Time spent in the center(s) for different treatments groups including different pretreatment group

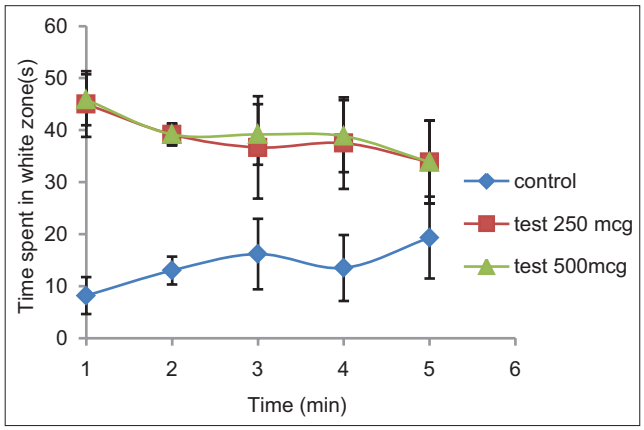

Fig. 2: Time spent in the white zone for different treatments groups including different pretreatment group

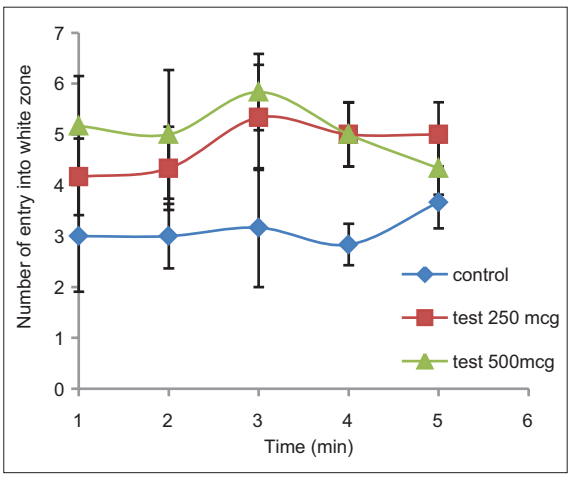

Fig. 3: Number of entry into the white zone for different treatments groups including different pretreatment group

DISCUSSION

The interesting fact evolving out from this model is that the higher and lower dose was not showing any significant difference for different parameters in white and black plus maze. Although this model is effective to find the difference between pretreated group, but the model cannot be effective to find the difference between lower and higher doses. Hence, this anxiety model can be used to find the anti-anxiety drug but when it was designed to find the effective dose of an anxiety drug, it was not so much accurate. This may be described in such a way that in this white/black plus maze model, the animals will show a state of anxiety when they belong to control group. When the pretreatment

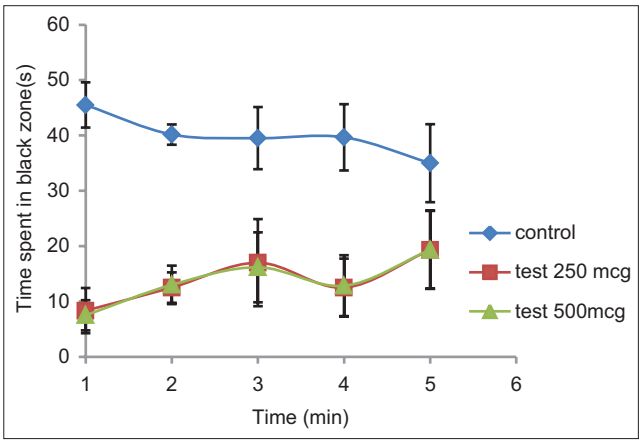

Fig. 4: Time spent in the black zone for different treatments groups including different pretreatment group

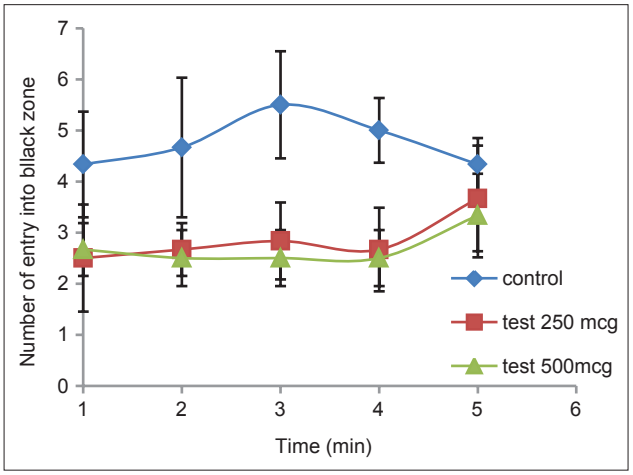

Fig. 5: Number of entry into the black zone for different treatments groups including different pretreatment group of higher and lower doses

dose is increased, the animal has changed its behavior but the behavior would not change vigorously when the dose was increased.

CONCLUSION

This experimental model was designed to find the effect of pretreatment on zebrafish behavior. The entire model was somehow successful when it was used to find the anti-anxiety effect of the drug midazolam. But the model has some limitations when the anti-anxiety effect was investigated at different dose of treatment. Overall, it can be postulated that this new model had some limited applications, but it would open a door for a new investigation in zebrafish behavioral research.

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REFERENCES

1. Zon LI, Peterson RT. *In vivo* drug discovery in the zebrafish. Nat Rev Drug Discov 2005;4(1):35-44.
2. Mueller T, Vernier P, Wullimann MF. The adult central nervous cholinergic system of a neurogenetic model animal, the zebrafish *Danio rerio*. Brain Res 2004;1011(2):156-69.
3. Panula P, Sallinen V, Sundvik M, Kolehmainen J, Torkko V, Tiittula A, *et al.* Modulatory neurotransmitter systems and behavior: Towards zebrafish models of neurodegenerative diseases. Zebrafish 2006;3(2):235-47.
4. Dooley K, Zon LI. Zebrafish: A model system for the study of human disease. Curr Opin Genet Dev 2000;10:252-6.
5. Norcross M, Mathur P, Enoch AJ, Karlsson RM, Brigman JL, Cameron HA, *et al.* Effects of adolescent fluoxetine treatment on fear-, anxiety- or stress-related behaviors in C57BL/6J or BALB/cJ mice. Psychopharmacology (Berl) 2008;200(3):413-24.
6. Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF, *et al.* Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav Brain Res 2009;205(1):38-44.
7. Bencan Z, Sledge D, Levin ED. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. Pharmacol Biochem Behav 2009;94(1):75-80.
8. Sackerman J, Donegan JJ, Cunningham CS, Nguyen NN, Lawless K, Long A, *et al.* Zebrafish behavior in novel environments: Effects of acute exposure to anxiolytic compounds and choice of *Danio rerio* line. Int J Comp Psychol 2010;23(1):43-61.
9. Fogarty JN, Vogel-Sprott M. Cognitive processes and motor skills differ in sensitivity to alcohol impairment. J Stud Alcohol 2002;63(4):404-11.
10. Colwill RM, Raymond MP, Ferreira L, Escudero H. Visual discrimination learning in zebrafish (*Danio rerio*). Behav Processes 2005 31;70:19-31.
11. Arthur D, Levin ED. Zebrafish (*Danio rerio*). Anim Cogn 2001;4*:*125-31.
12. Guo S. Linking genes to brain, behavior and neurological diseases: What can we learn from zebrafish? Genes Brain Behav 2004;3(2):63-74.
13. Drapier D, Bentué-Ferrer D, Laviolle B, Millet B, Allain H, Bourin M, *et al.* Effects of acute fluoxetine, paroxetine and desipramine on rats tested on the elevated plus-maze. Behav Brain Res 2007;176(2):202-9.
14. Serra EL, Medalha CC, Mattioli R. Natural preference of zebrafish (*Danio rerio*) for a dark environment. Braz J Med Biol Res 1999;32(12):1551-3.
15. Speedie N, Gerlai R. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). Behav Brain Res 2008;188(1):168-77.
16. Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. Neurosci Biobehav Rev 2005;29(8):1193-205.
17. Lapiz-Bluhm MD, Bondi CO, Doyen J, Rodriguez GA, Bédard-Arana T, Morilak DA. Behavioural assays to model cognitive and affective dimensions of depression and anxiety in rats. J Neuroendocrinol 2008;20(10):1115-37.
18. Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. Physiol Behav 2007;90(1):54-8.
19. Dundee JW, Halliday NJ, Harper KW, Brogden RN. Midazolam. A review of its pharmacological properties and therapeutic use. Drugs 1984;28(6):519-43.
20. Bolon M, Bastien O, Flamens C, Paulus S, Boulieu R. Midazolam disposition in patients undergoing continuous venovenous hemodialysis. J Clin Pharmacol 2001;41(9):959-62.
21. Longhi R, Corbioli S, Fontana S, Vinco F, Braggio S, Helmdach L, *et al.* Brain tissue binding of drugs: Evaluation and validation of solid supported porcine brain membrane vesicles (TRANSIL) as a novel high-throughput method. Drug Metab Dispos 2011;39(2):312-21.
22. Praskova E, Voslarova E, Siroka Z, Plhalova L, Macova S, Marsalek P, *et al.* Assessment of diclofenac LC50 reference values in juvenile and embryonic stages of the zebrafish (*Danio rerio*). Pol J Vet Sci 2011;14(4):545-9.
23. OECD. Guideline for Testing of Chemicals (No 203), Fish Acute Toxicity Test. Paris, France: OECD; 1992.