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Effects of anxiolytic drug as pretreatment therapy for anxiety of zebra fishes induced by the exposure of unfamiliar environment

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Abstract:

The research investigation was designed to find the behavior of zebra fish when it was exposed into unfamiliar atmosphere. The animals were divided into three different groups containing 6 zebra fishes. After pretreatment with high and low dose of Midazolam, two groups were undergone an exposure of unknown environment by immersing into novel dive tank. The last group of fishes as control group was immersed into the dive tank for 5 minutes without any pretreatment group. Induction of the anxiety was observed in the form of different parameters like number of cross to middle, number to cross upper 2/3rd , time spent in middle half (s), time spent in upper 2/3rd of the tank, total number of turns and latency time to enter middle half. The results illustrated that the improvement of anxiety disorder was clearly evident when the pretreatment of the animals with lower and high doses was done. It was also evident that higher dose a pretreatment significantly improved the anxiety as compare to lower doses. Overall the model can be described as a distinct behavioral method tool that is highly sensitive to find the proper drug and dose for the treatment with anxiolytic compounds.

Key Words: Novel Dive Tank, Zebra Fish, Midazolam, Stress.

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1. Introduction

Animal models based on zebra fish was recently used for investigation of the anxiety, depression, neurotoxicology and other model of behavioral disorders that are a contemporary research topic in translational pharmacological research[1-8]. The advantage of zebra fish over other species can be attributed as its flexibility, easy handling capability, resembling to human being and less cost effective[9-12]. Although nervous system of the zebra fish are quite simpler than that of human being and mammals but the zebra fishes generally display a complex behavior expression which are similarly modulated like mammals by different kind of central neurotransmitter system. In addition, this small fish was showing a reliable reproducible response to different kind of neurophramacological and biological treatment which resembles same aspect of human complex neurobehavioral characteristics. Anxiety disorders are quite common in different psychiatric conditions that will not only effect the emotional behavior but also it affect the cognitions attributes of the common people[13-15]. Anxiety is highly related to different kind of behavior response that can be reproduced in zebra fish models [16]. Here we have selected such a model which was described in literature as the as the name of novel dive fish model [17]. Here we designed and utilized a novel environmental paradigm where the fishes were put into an unfamiliar environment that induced anxiety among the zebra fishes. The dive tank model induced an anxiety which made the zebra fishes to remain in the bottom contact of the novel tank. This test was helpful to evaluate a new investigative model to find the effective dose of pretreatment of the anti anxiety treatment of zebra fishes. In addition, our research investigation was also designed to find the efficacy of Midazolam for the treatment of anxiety disorder of zebra fish which is originated due to exposure of unfamiliar environment that was imposed on zebra fishes by immersing them into the novel dive tank [18-21]. Finally, it can be postulated that this research experiment can be translated into a new paradigm of investigation on behavioral research studies where the anxiety was generated due to fear, stress in unfamiliar situation faced by the human beings in their life.

2. Materials and Methodology

2.1 Animals

The experiment zebra fish was procured from local commercial supplier (Blu Aquarium, Jayanagar and Prabu Aquarium, Pipeline road, India). All the zebra fishes were healthy and average weight around 30.8±. All the experimental zebra fishes were put in to an acclimatization main tank for minimum 3 weeks before starting the experiment.

2.2 Reagents

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

2.3 Experimental Design

A novel dive tank was designed in such a way that it was like trapezoidal structure where the sides of the trapezoids were lesser than as comparing to upper section of the dive tank. This model had produced stress condition of animals when the fishes were

immersed into the dive tank. The dive tank was divided in to 3 sections by drawing line with the marker outside the tank to help the observation of the animals. The tank was fixed on a black counted top with exposed white back ground against its back wall to enhance contrast for video recording by using digital camera. All the fishes in the dive tank was observed and monitored for 5 minutes to evaluate different behavioral parameters including number of cross to middle, number to cross upper 2/3rd , time spent in middle half (s), time spent in upper 2/3rd of the tank, total number of turns and latency time to enter middle half. The dive tank was designed according to the previous experiment of zebra fish behavioral studies conducted by Sackerman 2010 et al.

Before starting the experiment all the animals were divided in to 3 groups each contains 6 animals. One group animal was directly immersed in to the novel dive tank without any pretreatment of drug. Other two groups were exposed to a high concentration and a low concentration of Midazolam respectively in a 1 L beaker individually before immersion of the animal into the anxiety inducer dive tank. The pretreatment of zebra fishes was conducted for 5 minutes for both high and lower doses of Midazolam. The treatment of the animals including pretreatment of zebra fish was done for 5 minutes. During these 5 minutes all the behavioral parameters including number of cross to middle, number to cross upper 2/3rd , time spent in middle half (s), time spent in upper 2/3rd of the tank, total number of turns and latency time to enter middle half was recorded for further experimentation. This parameter was expressed in the form of scientific data which represented the anxiety state of the zebra fishes. The whole experimental study was illustrated in schematic diagram as represented below.

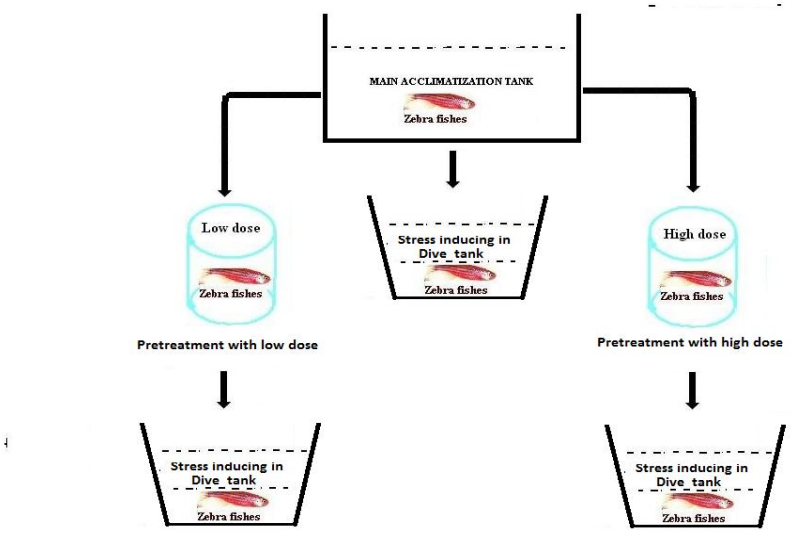

Fig 1: Schematic diagram of zebra fishes treatment including different doses of pretreatment of high and low doses

2.4 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 guide line [22-23].The experimental animals were immersed into different concentration of drugs for 96 h to find the mortality rate. 50% animals of total

population were found to be died in the concentration of 5mg/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.

2.5 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 curve in terms of score intensity, latency period and wash out period were represented as mean ± SE followed by one way ANOVA test and post hoc test. Student 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.

3 Results

The dive tank was divided into 3 parts. 3 parts can be named as upper, middle and lower section. Fig 2 represents the number of cross performs by the zebrafish from lower section to middle section. When the zebra fish was relaxed and free from stress, number of cross to middle zone from lower zone was increased. The results illustrated that when the animals were only immersed into the dive tank performed less number of crosses to middle zone as compare to the animals which were undergone Midazolam pretreatment($p=<0.05$). Although high dose of the Midazolam which was used as pretreatment increased the number of crosses as that of lower dose but the result was not statistically significantly $p=>0.05$). The inference of the result concluded that Midazolam pretreatment increased the number of cross to middle but the increment of the dose did not affect so much in the parameters of cross lower to middle by zebra fishes.

The interesting factor here it was observed that when time increased the number of cross to middle half increased steadily for all treatment group. The results illustrated that all the zebra fishes animals gradually became fearless and stress free when the time lapsed.

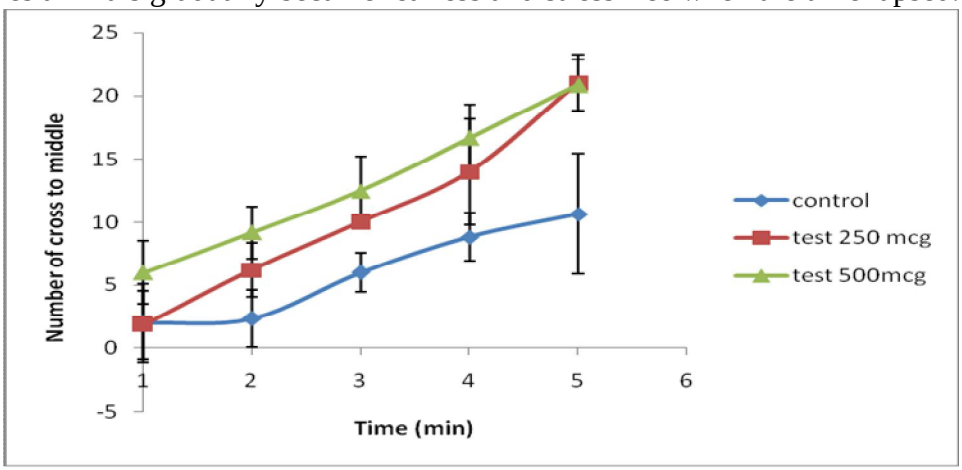

Fig 2: Number of cross to middle by zebrafish for different treatment groups including different pretreatment group.

When the animals are in the stress and fear, number of cross upper to two thirds from the lower zone decreased significantly. Fig 3 represents the number of cross upper to two thirds from the lower zone by experimental zebra fishes in different treatment groups. In case of control group the number of cross upper to two thirds increased gradually from time one to five minutes. The treatment of control and test group was quit same as the number of cross to upper two thirds increased gradually starting from time one to five minutes. In case of high test group the zebra fish has crossed the upper two thirds more than as compare to lower test group and control group and the result was quite statistically significance p=<0.05.But when the results was compared in between lower test group and control group the difference was found very less which was statistically insignificant (p=>0.05). So the results can be described that when the animals were pretreated with high doses the number to cross upper two thirds increased significantly but lower was not much effective for improvement of number of to cross upper two thirds. Here it was also found that number to cross upper two thirds increased gradually during time of experiment.

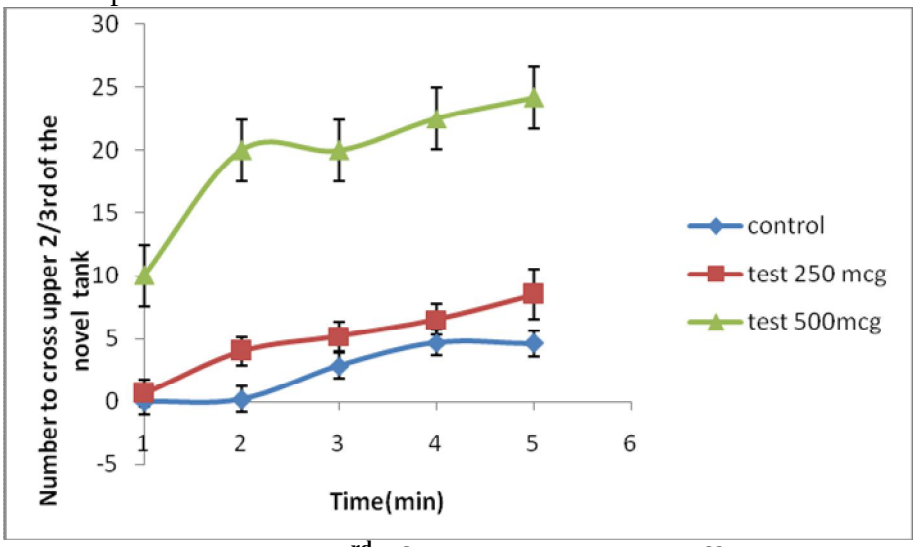

Fig 3: Number to cross upper 2/3rd of novel test tank by different treatment groups of zebra fishes during the time of experiment.

Fig 4 illustrated time spent in the middle half at different duration of time. The time spent in middle half indicated the relaxation of these animals from anxiety, fear and stress. The control group of animal significantly spent lesser time as compare to test of higher dose and lower dose. During first four minutes the time spent in middle half significantly differ as in case of lower and high dose .After the time of four minutes the time spent in the middle half has no difference in case of lower and higher doses. The results clearly indicated that high dose of Midazolam improved the time spent in middle half instantly but after a certain period time spent in middle half did not increase significantly. But the lower doses increased the time spent in the middle half very steadily and after certain time lower dose had the same efficacy as that of higher doses. Time spent in middle half by the all zebra has increased gradually from time one to five minutes. This also indicated that at the initial stage animals were in stress condition but when the time elapsed the animals became stress free irrespective of any treatment dose.

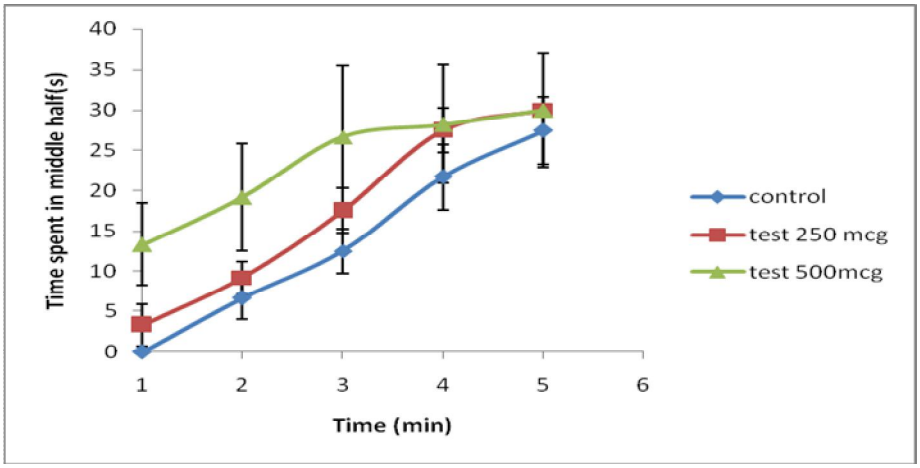

Fig 4: Time spent in middle half(s) by zebra fishes in a novel test tank for different treatment groups during the experiment

Fig 5 was plotted by the time spent in the upper two thirds of the tank at different time interval. The result showed that time spent in the upper two thirds of the tank was reflecting the animals behavioral status when there in stress condition. The higher dose of animal significantly spent much more time as compare to lower dose and control group (p=<0.05). the lower dose of the animal was also spending much more time during the first four minutes as compare to normal dose (p=<0.05) but at time five minute the time spent in seconds by the fishes in upper two thirds of the tank was quite same and difference was statistically insignificant (p=>0.05). In all cases the improvement of fear and stress was observed in final stage of the experiment.

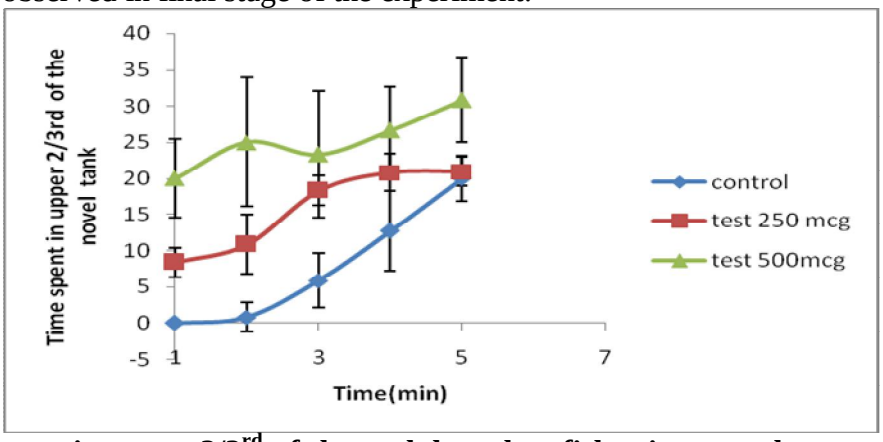

Fig 5: Time spent in upper 2/3rd of the tank by zebra fishes in a novel test tank for different treatment group.

Fig 6 was plotted as the number of turns during different time interval of the experiment. At initial time of experiment the number of turns in all cases was quit same and the difference was statistically insignificant (p=>0.05) but when the time progressed the number of turns increased after pretreatment of zebra fishes with different dose of Midazolam. In case of higher test group the number of turns starting from time 2 to 5 minutes was significantly higher than as compare to lower and control group. In addition lower group of treatment also produced a significant difference in number of turns as that of control group of animals during the time interval of the experiment starting 2 to 5

minutes. It should be remembered that improvement of number of turns always represented that the animals were free from stress and fear.

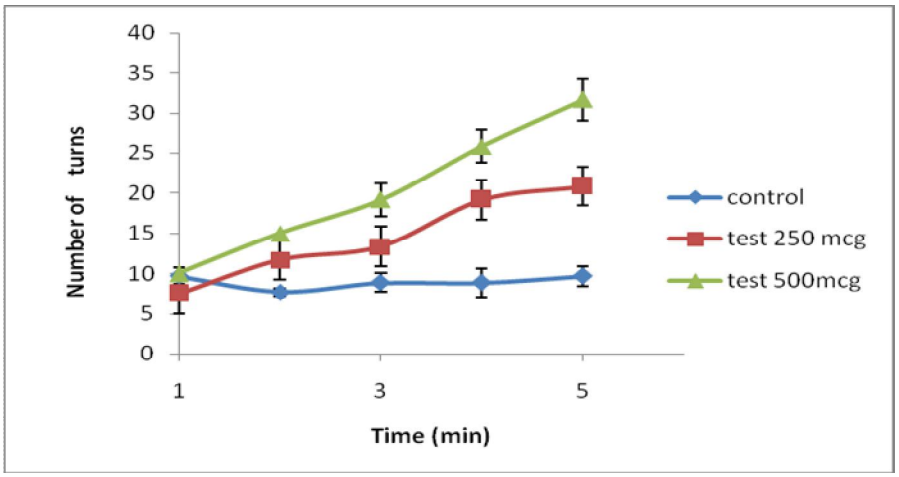

Fig 6: Number of turns of zebrafish by zebra fishes in a novel test tank for different treatment group.

In Fig 7 the latency time to enter into middle half was plotted against different kind of treatment group. If the animals were stressed it would take more time to enter first time in to middle half. The results indicated that the latency time to enter middle half was significantly lesser in case of higher doses than that of lower dose and higher dose.
The latency time to enter middle half in case lower dose was quit significantly lesser than that of control group ($p=<0.05$).

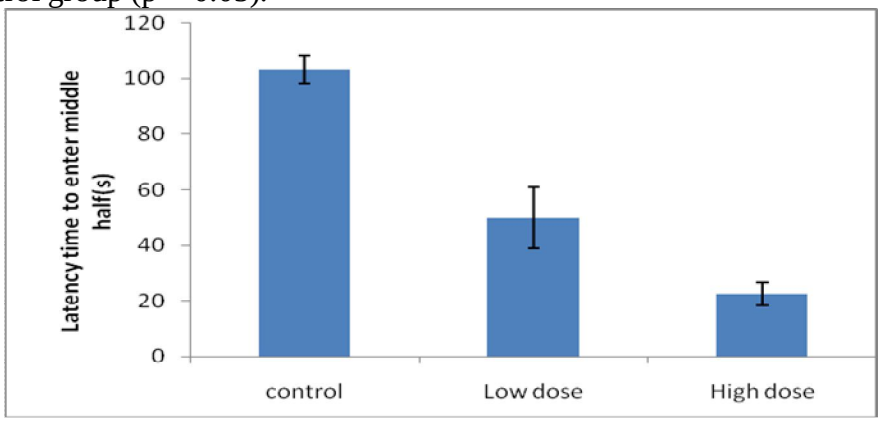

Fig 7: Latency time to enter middle half(s) of zebrafish in a novel test tank for different treatment group.

4 Discussion

The overall results can be concluded that all the animals became stress free when the experiment progressed instead of using different kind of doses. These results can be described that when the time progressed animal became habituated with the atmosphere and gradually became stress free, so time was an important factor to reduce the stress which was induced by putting the animal in the novel dive tank. Another point was observed in our study which was the effect of treatment dose of different concentration on the behavioral status of pretreatment zebra fishes. It was clearly evident that when the dose was increased up to certain level the results always showed better therapy than

compare to lower dose and control group. From this data it can be expected that a higher dose of pretreatment of Midazolam can induce an anti anxiety behavior on zebra fishes which showed resistance to anxiety when the animal immersed in to the novel dive tank. In same way the animals with lower treated doses was showing better results in compare with control group but the results was not always found better in different parameters including number to cross upper 2/3 and time spent in middle half. These results indicated that lower dose treatment was not effective to treat the animal for its overall treatment of stress and fear. So the entire results can be described in such a way that higher concentration of treatment with Midazolam produced a brain concentration in such level that they treated the animal in overall aspect of different kind of anxiety behavior.

Conclusion

The entire experiment was investigated to find the pretreatment of Midazolam on induced zebra fishes in anxiety induced zebra fishes which were executed by the use of dive tank. The experiment explores a new investigative model which will be helpful for future research experimentation on different kind of behavioral studies in zebra fishes.

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