

Locomotor Activities of Adult Zebrafish (*Danio rerio*) Under the Influence of Pranic Energy: Controlled Study

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Abstract

Movement plays an important role throughout the lifespan of every moving organism. In scientific experimentation, Zebrafish (*Danio rerio*) is a popular model organism due to their genetic attributes similar to humans and rapid development. Pranic energy comprises the natural method of projecting prana (Vital energy) which keeps the plants and animals healthy and alive. The present study aims to investigate the impact of pranic energy on locomotory performance of adult zebrafish. Controlled experimental design has been used in this study. Pranic group received the Pranic healing techniques for fish, food, and water to reinforce the energy in them, non pranic treated fishes were referred as the control group. Locomotor behaviour of individual fishes was performed by exposing in an open area for 5 minutes and parameters including average speed, mobile speed, total distance travelled, mobility rate and exploratory rate were measured and analysed by Tox-Track tracking software. Pranic treated zebrafishes (46) had increased average speed of 8.77 mm/s, mobile speed of 8.15 mm/s and total distance travelled of 32.050m and the results were found to be significant ($p < .001$), when compared to control group (43). Thus pranic energy helps in enhancing the locomotory performance of adult zebrafish.

Keywords: Life Force, *Danio rerio*, Behaviour, Locomotion, Speed, ATP.

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INTRODUCTION

Pranic Healing is an ancient science and art of energy healing through vital energy or Prana. As a complementary therapy, Pranic Healing was found to improve the condition of mild and moderately depressed patients (Rajgopal et al 2018), to improve the lung function (Mahesh et al 2017), anxiety disorders, immune power, headache, arthritis, asthma (Sui, 2015). Energy healing is supposed to work by manipulating human subtle energy (Prana) for the proper functioning of the physical body. Out of many energy practices including reiki (Kelly, 2000), acupuncture (Madsen et al 2009), acupressure (Cross and Charman, 2006), pranic energy among others. Pranic Agriculture works on the holistic concept of Eco-friendly farming system, consists of entire invisible subtle energy which is an essential and significant part of nature system (Prasad & Jois 2020). The life force which keeps the body strong, healthy and alive is called prana (Sui, 2015). According to Sui (2015), Prana is universal, one can feel, scan and interact with this energy. Every object including plants, animals and trees are enclosed by an energy field called bioplasmic body or aura consisting of prana (Sui, 2015).

Prana is also referred to as ki or chi. Sui (2015) specifies that there are 3 major sources of prana namely air prana, solar prana and ground prana. Water absorbs prana from ground, air and sunlight. Plants and trees absorb prana from the ground, air, sunlight and water, animals obtain prana from sunlight, air, water, food, and ground. Pranic energy can be projected by anybody with some familiarity of pranic healing techniques. Pranic treatment can be applied to plants to enhance their growth, yield, and improve their physical, nutritional and biochemical qualities (Krell & Riebessel, 2015). Pranic energy significantly increased the germination of green gram seed, compared to control group (Jois et al 2016). Antioxidant and polyphenol content of cucumber were also improved significantly when pranic energy was projected (Keerthika et al 2016).

Zebrafish (*Danio rerio*), a small cyprinid family teleost fish native to the river Ganga of East India, Bangladesh, Nepal and Burma (Arunachalam et al 2013). Adults have been observed abundance in well-vegetated shallow regions, pools, drainage, paddy fields and at the slow-moving canals. The species is identified by a small size (4-5cm), cylindrical body, alternating black and white stripes with distinct colour pattern (Menke et al 2011). Animal models play a major role in the scientific investigation for studying their behaviour and (Patho) physiological mechanism. Zebrafish as a vertebrate model, studies on zebrafish have steadily increased, in the fields of biomedical science, including toxicology, pharmacology, drug discovery, genetics, behavioural neuroscience (Fadool & Dowling 2008; Sison & Gerlai 2010), due to its small size, the high degree of physiological, morphological and genetic homology to humans (Kalueff et al 2013), fully sequenced genome shows orthologous corresponds to 82% of diseases related genes in humans (Howe et al 2013), rapid and external embryonic development, clear observation of well-characterized behaviour, easy maintenance with a large stock of fish (Norton & Bally Cuif, 2010). The assessment of adult zebrafish in behaviour neuroscience has increased in recent decades because of its unique interface between intrinsic and extrinsic forces which determines the

organism survival and health (Macphail et al 2009, Little & Seebacher, 2013). Several studies are carried out on the zebrafish behavioural development of sensory and motor functions (Fero et al. 2011; Drapeau et al 2002). However human behaviour linked with a psychiatric disorder, stress condition and drug addiction has many interrelated genetic and environmental causes, understanding these disorder is technically challenging. Hence zebrafish is gaining popularity as a model organism in behavioural research and many protocols have been developed to measure the locomotion, learning, anxiety, memory, social preference, rewards etc for better understanding human disease (Tegelenbosch et al 2012). The present study aimed at investigating the impact of pranic energy on locomotory performance of pranic healed zebra fishes against the control.

MATERIALS AND METHODS

Specimen and Housing

All experiments were carried on wild type AB genotype Zebrafish (*Danio rerio*), larval stage 30-40 dpf (days post-fertilization) were procured from a certified breeding centre in Chennai, India. Fishes were allowed to grow until 3 months (adult phase) in a well-oxygenated circulation aquarium tank filled with dechlorinated tap water. The fish tank was maintained at a temperature of 28°C ($\pm 1^\circ\text{C}$), pH ranged between 8 ± 0.4 on a photoperiod of 14:10 light and dark cycle with the continuous filtration and half of the water was replaced after every 15 days. Fishes were fed twice a day (once in morning and early evening) with freeze-dried blood worms and diet was supplemented with micro pellet (Spectrum Brands Company, Germany), At most care was taken to all fishes.

Experimental Design: Controlled experimental design has been used in this study.

Sampling: Two identical aquariums, containing control group (43) and pranic group (46) adult zebra fishes were used for this study.

Behavioural Analysis: Zebrafish from larval to adult stage (3 months) were reared as described above. The pranic group fishes received pranic healing techniques for 3 days/ week from its larval to adult stage by a pranic healer. Pranic healing techniques were applied to fish, food and aquarium water. Locomotion activities were performed, to measure the swimming pattern of pranic group adult fishes against the control group. The swimming pattern of each fish was recorded for 5 minutes by placing individual fishes in an open arena, a rectangular transparent glass tank (50x20x20cm) under constant room temperature and specific light condition. Before recording all the fishes were allowed to accommodate to the novel environment for 1 minute. Locomotory activities were video recorded continuously by a camera which was fixed above the arena, to start and monitor the experiment video the camera was connected

to the PC (Egan et al., 2009). During locomotion activity, average speed (mm/s), Mobile speed (mm/s), Total distance traveled (mm), exploratory rate (%) and mobility rate (%) were calculated from the acquired coordinates (Kalueff et al 2013), additionally, we analysed the locomotory trajectories of each fishes. All the recorded parameters were analysed by Tox- Track tracking software (Rodriguez, et al 2018).

Statistical Analysis:

Analysis of variance (ANOVA) was used to analyse the locomotor activities of adult zebra fishes including average speed, mobile speed, and total distance travelled. Mann-Whitney U test for mobility rate and exploratory rate to compare the differences between groups. The difference was considered significant at $p < .001$.

RESULTS

Average Speed

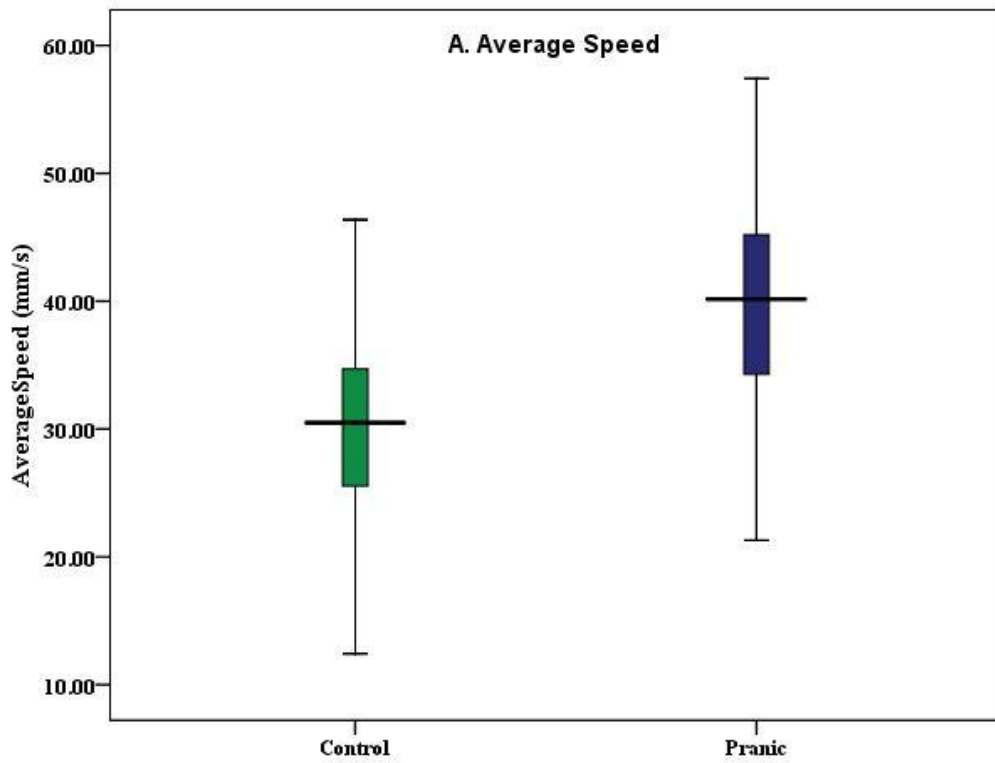
Average speed was measured by calculating total distance travelled over the total duration of time which interprets the neurological phenotypes and general motor function of zebrafish. Average speed was found to be higher in pranic treated group (39.38 mm/s) than the control group (30.61mm/s). It was observed that pranic group had 8.77 mm/s more average speed compare to control group ($p < .001$) (Figure 1A). Our findings were relevant to earlier research (Sarasamma et al 2019) behavioural impairments caused by chronic exposure of C70 nanoparticles on adult zebrafish were average speed shown 40mm/s on exposure to the novel environment for 5minutes.

Mobile Speed

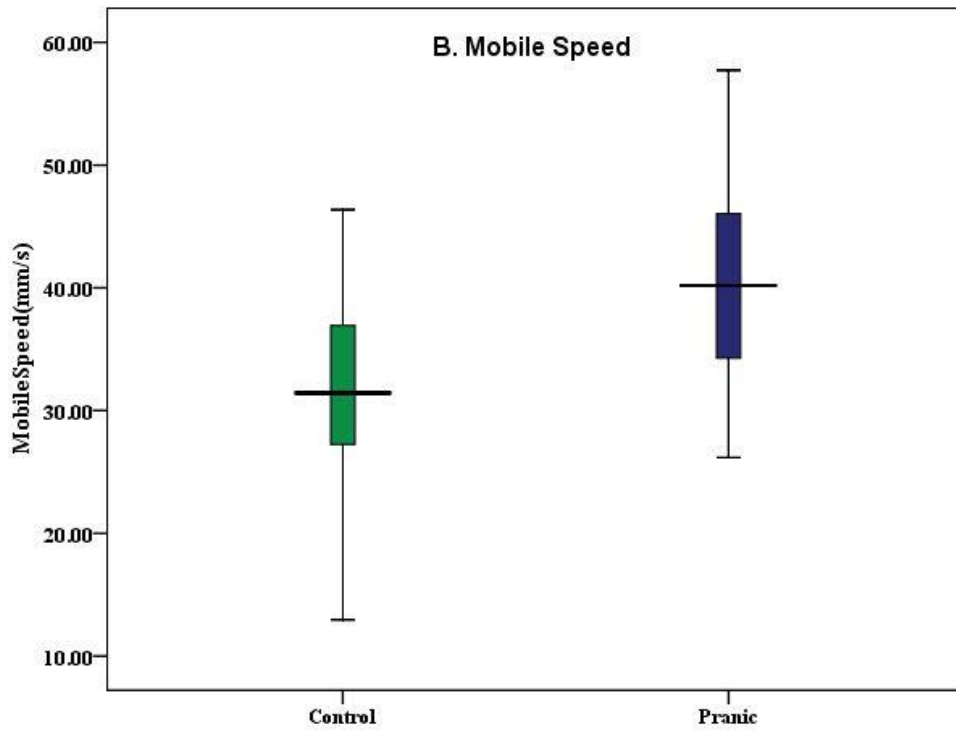
Mobile speed of adult zebrafish was analysed in an analysis of variance with pranic treated group (Pranic treated v/s Control) as the independent variable. The mobile speed was greater in the pranic treated group (39.69 mm/s) than control group (31.54 mm/s), it was found that pranic group had (8.15 mm/s) speed than the control group ($p < .001$) (Figure 1B).

Total Distance Travelled

Total distance travelled were analysed by calculating the total distance of zebrafish travelled when a fish introduced to a novel environment, which reflects the general motor function of the zebrafish swimming. Total distance travelled in adult zebrafish was found to be higher in pranic treated group 121.180mm than the control group 89.130mm, there was a significant difference ($p < .001$) between the pranic and control group (Figure 1C), hence pranic group adult zebrafish has travelled 32.050 mm more distance than the control group.



(A)



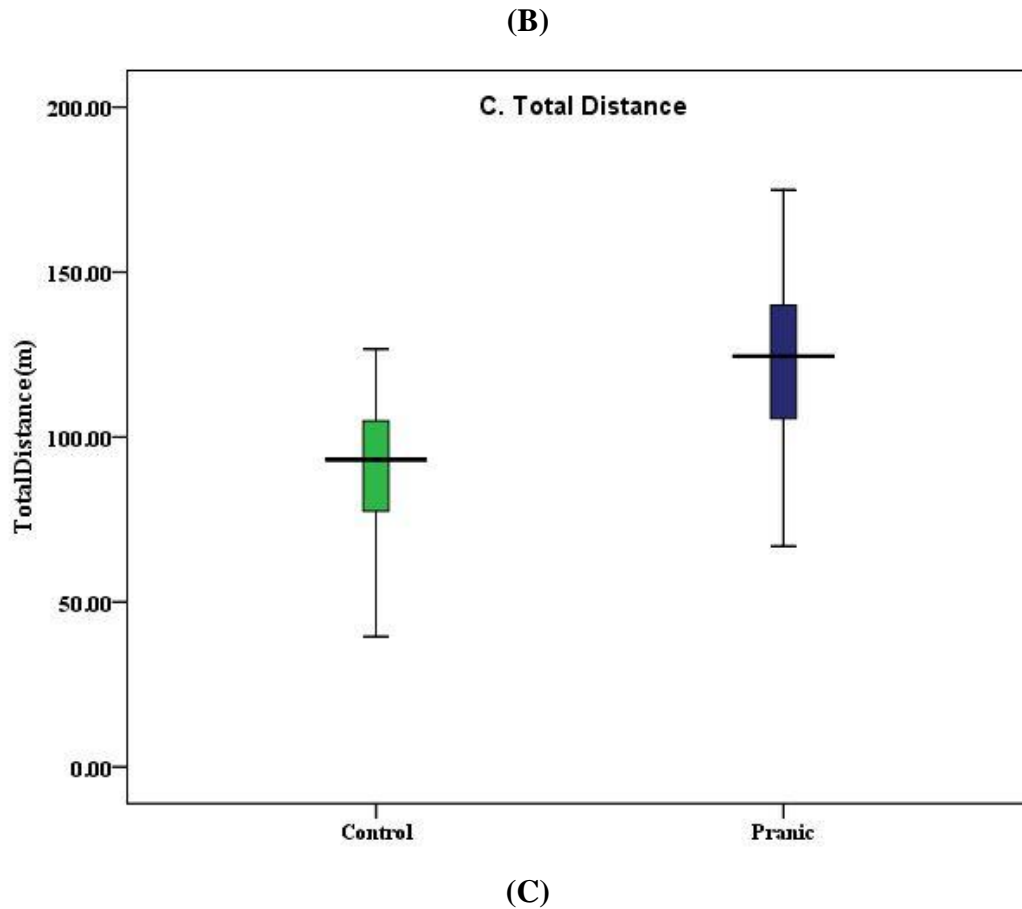


Figure 1. Behavioural Comparison of Average speed (A), Mean speed (B) and Total distance travelled (C) between control and pranic treated zebrafish.

Mobility Rate

Mobility rate correlates with speed and distance travelled which indicates the percentage of zebrafish travelled more easily and freely when it is introduced into the novel environment, median of pranic treated adult zebrafish had significantly higher rate of mobility (mean rank = 49.09%), compared to control group (mean rank = 40.63%), ($U = 801.000, p = .021$). Hence pranic treated zebrafish had 8.46% more mobility rate compared to the control group (Figure 2A).

Exploration rate

Exploration rate related to, but not entirely dependent on anxiety and locomotory related parameter. It is measured by quantifying the ratio of zebrafish activity in different vertical and horizontal area of the tank. The median of pranic treated adult zebrafish had a significantly higher exploration rate (mean rank = 53.17%) compared to control group (mean rank = 36.26%), ($U = 174.700, p = .002$) (Figure 2B & 4).

Hence pranic treated zebrafish had 16.91% more mobility rate compared to the control group (Figure 2B).

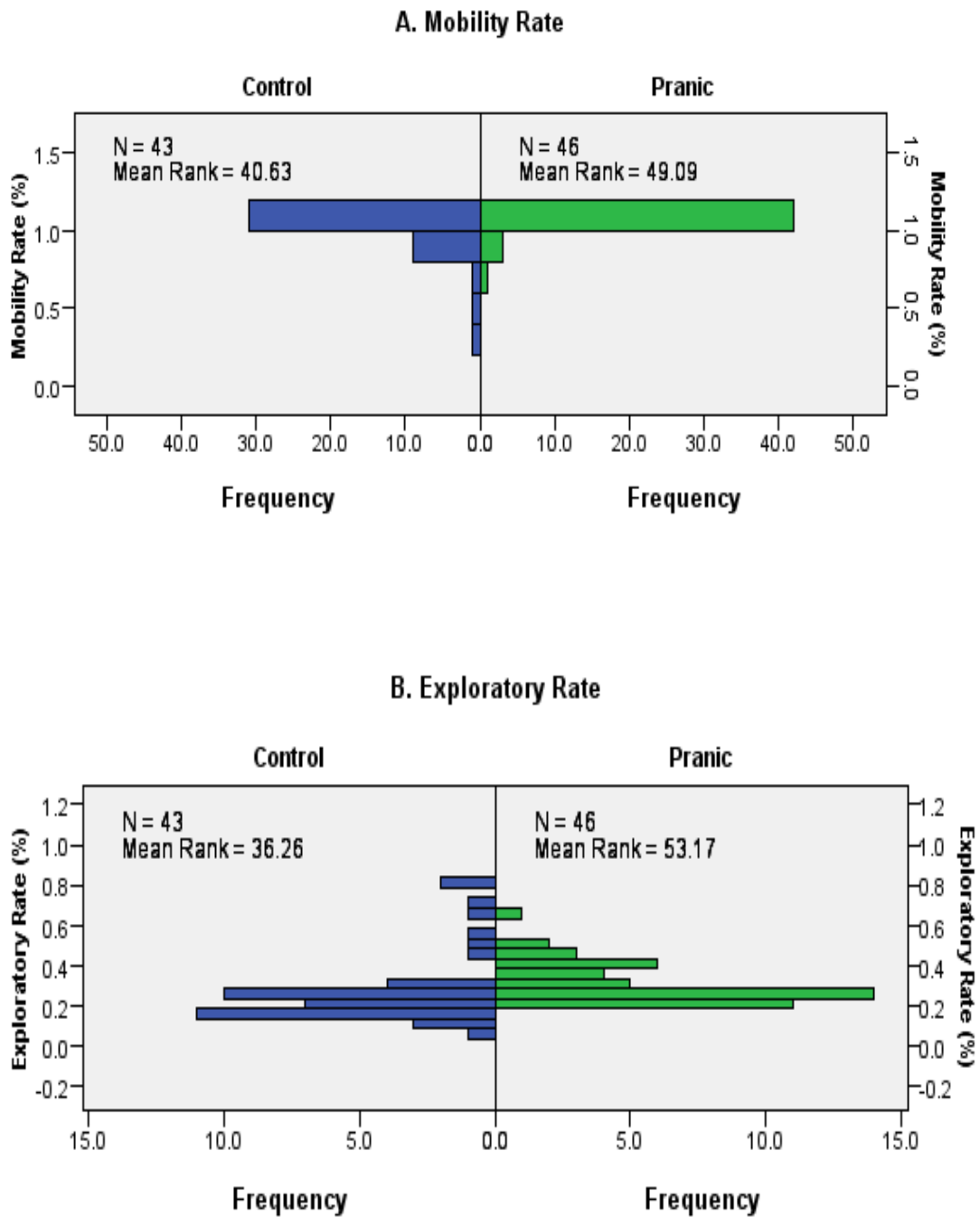


Figure 2. Behavioural Comparison of mobility rate (A) and exploratory rate (B) between control and pranic treated zebrafish.



Figure 3. Growth difference of aquatic plant between control and pranic group.

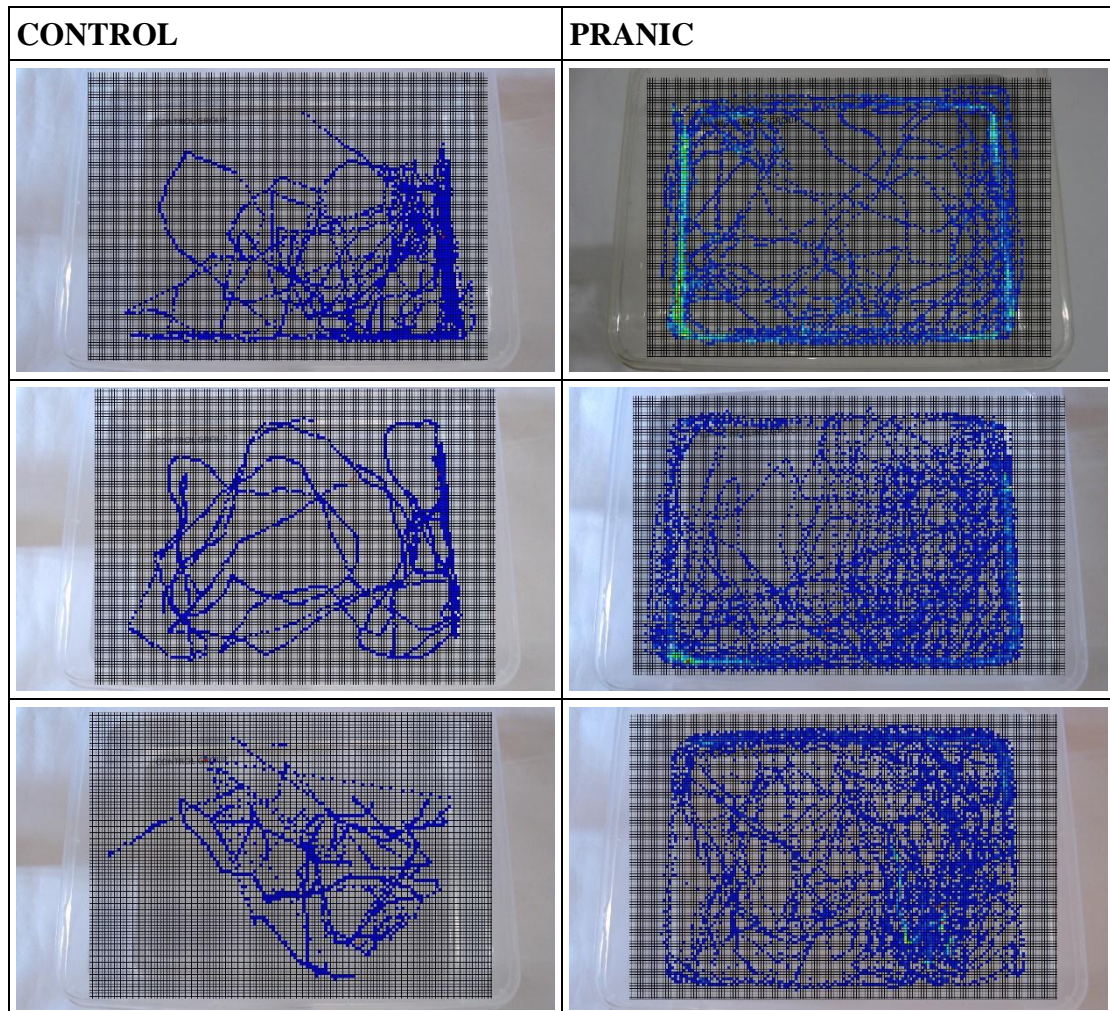


Figure 4. Locomotion trajectories of control and pranic treated zebra fishes.

DISCUSSION

In the present study, we analysed locomotory behavioural end points including Average speed, mean speed, total distance travelled, mobility rate and exploratory rate to evaluate the impact of pranic energy on the adult zebrafish. The most significant finding in the present study is the increased locomotory activities in pranic treated adult zebrafish. The movement being one of the characteristic features of every living organism which helps to move from one place to another. Locomotory behaviour plays an important role throughout the zebrafish lifespan including migration, feeding, schooling, Social preference, reproduction and predator avoidance activities (Husak et al 2006), thus reduced locomotory behaviour may affect the probability of survival in zebrafish (Zhang et al 2017). Motor behaviours are controlled by microcircuits in the central nervous system (Marder et al 2003; Kyriakatos et al 2011). Different types of motor behaviour are generated by a spinal cord through the coordination of microcircuits activity (Berkowitz et al 2010). Each type of behaviour varies in their speed, velocity, force and patterns of swimming, specific neurons are dedicated to specific motor behaviour (Fetcho and Mclean 2010; Robertset al 2010). Locomotion and muscle function directly depends upon the availability of sufficient energy (ATP) (Allen et al 2008). Earlier research on zebrafish larvae showed that deficits in the energy lead to change in their locomotory behaviour, thus every task performed by living organisms requires energy (Zhang et al 2017). In addition to that presence of high ATP/ADP ratio promotes Cell division, DNA synthesis and protein translation which is essential for zebrafish growth and development (Zhang et al 2017).

Pranic healing or pranic agriculture works on the energy field similar to Acupuncture, Reiki, Acupressure. Similar to humans, pets including fishes possess an energy body and physical body, among this energy body serves as the template for the physical body. For the physical body should be healthy, energy body has to be clear and balance. Once the energy body is out of balance or congested then the physical body will develop symptoms. According to Bai et al 2000, pranic energy enhances the ATPase activity which provides more energy to the cells during cell growth, division and differentiation. It should also be noted that application of pranic energy alters the structure of cellular organs like mitochondria which influences the metabolism by altering molecular structure of the cell, affects the nucleotide polymerization, gene expression and enzyme activity (Trivedi et al. 2015). In addition to that large proportion of magnetic fields are produced from the healer's hand during the projection of prana (Beseme et al 2018) these magnetic fields influence the enzyme activity by altering the pH of the solution (Strickland and Boylan 2010). Hence, pranic energy enhances the life force which keeps the body functional healthy and alive, probably application of this pranic energy influences the energy level of fish which results in an increased locomotor behaviour of pranic treated adult zebrafish.

It was observed that growth of aquatic plant in the aquarium was considerably higher in the pranic treated group compared to control (Figure 3). However, the presence of live plants in aquarium promotes the health of fishes by enough oxygen production, absorbing carbon dioxide and ammonia that fish produces, Competes with algae and

reduces algae growth, improves the quality of water and reduces the stress (Wersal & Madsen 2012).

Research on pranic energy is getting an increase in different disciplines including biology, health, agriculture, and Psychology (Prasad & Jois 2020). Earlier research on Chronic Obstructive pulmonary disease (COPD) patients, were a 6 minutes walking test was conducted, a positive trend in walking as well as improvement in heart rate, respiratory rate and blood pressure was noticed in pranic treated COPD patients (Mahesh et al. 2017). Similarly, studies on pranic agricultural revealed an increase in germination, seedling vigour and vegetative growth in pranic treated papaya than the control group (Prasad & Jois 2020).

CONCLUSION

Zebrafish treated with pranic energy has shown an overall increase in locomotory performance including average speed, mobile speed, total distance travelled, mobility rate and exploratory rate. It can be concluded that pranic energy played an effective role in increasing the locomotory activity during swimming. Further research is warranted to know the exact mechanism behind the increased locomotion in pranic treated zebrafish.

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