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ORIGINAL ARTICLE

Anti-Parkinson's Activity and Behavioral Assessment of *Luffa cylindrica* using Zebra Fish Model

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Abstract

Animal models play a central role in the biomedical and neurobehavioral research and for producing both experimental data and developing novel ideas. To understand the fundamental characteristics of physiological, behavioral and psychological problems researchers are constantly working to create new animal models. Due to the ethical considerations alternative animal models WERE proposed. Zebrafish (Danio Rerio) now became a popular unique animal model and widely used in neuropharmacology and biomedical research due to their high throughput amenability, screening potential for new therapeutic drugs. The objective of the current investigation was to screen anti-parkinson's activity and assessing of behavioral alterations in adult zebrafish followed by acute exposure to different pharmacological and herbal compound. Adult zebrafish of 3-4 months old were exposed to different concentrations of test drug (Luffa cylindrica), toxic drug (Paraquat) and the standard drug (Levodopa) for 15 mins. Individually each medication concentration and control were tested. Behavioral activity parameters were performed by using locomotor activity, novel tank diving test and light and dark transition test and catalepsy were recorded for each animal during exposure period. Zebrafish exposed to test drug substance and standard drug displayed behavioral change that were estimated. According to the results, adult zebrafish exposure to test group caused the behavioral changes like recovery of latency to travel from one point to another as compared to toxic group and exhibited significant reversal of the anxious behavior as compared to toxic group. As a result, adult zebrafish can be used as an alternative model for evaluating novel chemical entities on behavioral activity.

Keyword: Zebrafish (DANIO RERIO), *Luffa cylindrica* leaves, Novel Tank diving Test, Light and Dark Transition Test. Anti-Parkinson's activity.

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Introduction

Parkinson's disease: Parkinson's disease is a neurodegenerative disorder. It has clinical characteristics like rigidity, tremors, bradykinesia and postural instability (C.A.Davie 2010). It chiefly affects the nervous system and different parts of the body, which are controlled by the nerves. It was first identified and described by the scientist named James Parkinson in the year 1817

(Parkinson J 1817) and is characterized as a shaking palsy with both motor and non-motor features. The people who were affected with Parkinson's disease are usually at the average age of 60. There are mainly three types of Parkinsonism - Idiopathic Parkinsonism, Vascular Parkinsonism and drug induced Parkinsonism. It can be diagnosed based on its symptoms, detailed medical history and physical examination.

Cure is by the improving physical activity which is a good way to treat patients with Parkinson's disease (SM Hague et al., 2019). Aerobic exercise also helps to delay or prevent the onset of the disease. Diagnosis of Parkinson's disease is based on its symptoms, medical history, review of a neurological and physical exam. Medication, physical activity and therapies are the main treatments to help manage the Parkinson's disease. Surgery like Deep Brain Stimulation (DBS) is a neurological procedure that uses implanted electrodes and electrical stimulation to treat movement disorders associated with Parkinson's disease (S J Groiss et al., 2009). To treat people with Parkinson's disease the approved drug FosLevodopa – FosCarbidopa (also called produodopa) is used for movement related symptoms. Produodopa is delivered within 24 hours. Levodopa is most effective drug for the treatment of symptoms of Parkinson's disease. It particularly helps the people, who are with slowness of movement which is caused by bradykinesia (Jankovic J. 2008).

Tremors can occur even at rest. Other related symptoms like muscular stiffness difficulty in standing, walking, difficulty with bodily movements, involuntary movements, muscle rigidity, problems with coordination, rhythmic muscle contraction, slow bodily movement are also seen. Some rare symptoms like early awakeness, nightmares, fatigue, and dizziness can also be noticed. Anxiety, distorted sense of smell, dribbling, poor balance, jaw stiffness, blank stare, depression, fear of falling and weight loss were also reported in Parkinson's disease.

Herbal medicines and their derivatives have been incorporated into traditional medicine. Approximately 75% of the population in emerging nations receive herbal medical health care, compared with over half of the population. A review of the literature indicated the potential benefits of the use of a number of plant preparations. An attempt has been made to document Luffa cylindrica for its anti-Parkinson's effect and also its behavioral changes.

Materials and Methods

Plant material: Luffa cylindrica is an important edible medicinal plant which belongs to the family of *cucurbitacea*. *Luffa cylindrica* is a large climber plant with thick and strong stem (Indumathy R et al., 2011). The seeds of this plant is 1 to 2 cm long and width is 0.8 cm and they are smooth or oval shape. The immature seeds are white in color, whereas mature seeds are dark brown or black in color having a shallow root system. The leaves are very large in size i.e. 10 to 20 cm in long and 9 to 21 cm in width. *Luffa cylindrica* is commonly called as Sponge gourd. With the help and support of the climbing tendrils, the cultivation of plants in larger pots or direct into greenhouse border was done. Propagation of *luffa cylindrica* involves sowing of seeds at 21 to 24°c early in the spring season. These are annual plants and their entire life span will be completed in a single growing season. *Luffa* grows vigorously in hot and humid conditions and they need full sunlight and moist soil with high amount of minerals to grow. Alkaloids, steroids, flavonoids, glycosides, are the phytochemicals present in luffa *cylindrica*. This plant is taken in the view of inhibiting and treatment of cold and nasal sinus problems.

Women mostly use the *luffa cylindrica* plant to restore the absence or irregular menstrual periods (Tsai Hua Kao et al., 2012). These plants mostly grow in the countries like India, China, Nepal, Bhutan, Bangladesh and Vietnam. It takes 90 to 120 days to grow the fruit and it is also eaten as vegetable. Common diseases that the plant will get affected are Downey mildew, powdery mildew, alternating slight and the angular leaf spots, which affects the leaves of *luffa*.

The vitamins like riboflavin, niacin, vitamin-c and essential amino acids are present in this *luffa* gourd (M P Swetha et al., 2016). It is also rich in vitamins, minerals and antioxidants. It is having a variety of health benefits like helps in easy digestion, reducing inflammation and boosting the immune system. The seed oil of luffa cylindrica is used to cure chest pain, backache, infection of the intestine and inflammation and pain in the joint and muscles. It is also used to cure boils. Luffa fruit is rich in phosphorus, potassium, vitamin-A and vitamin C. The luffa gourd which is largely grown and tastes bitter should not be eaten, as it shows some poisonous properties.

Animals: Zebrafish were obtained from local aquarium shop, Hyderabad. Adult wild-type AB strains of zebrafish (3-5cm) of both the sexes of 4-6 months old were used (Spence R et al., 2020). The fishes were habituated to the laboratory conditions for 14 days and housed in 50 L tank filled with unchlorinated water at the temperature of 28 ± 2°c with constant filtration and aeration. Density of 5 fishes per liter was maintained. Animals were kept in laboratory conditions and were fed once a day with aquarium food.

Zebrafish is a fresh water aquarium fish, which can grow up to 1.5 to 2 inches long. It is named so, as it contains longitudinal stripes on it, which extend up to end of the caudal fin (Hill AJ et al., 2005). Shape of zebrafish is in fusiform and laterally compressed with its mouth directed upwards. The scientific name of zebrafish is Danio rerio. It belongs to the family-cyprinidae. Zebrafish is most widely used vertebrate model in the scientific research and studies. It was discovered by George streisinger. Small size, in vivo, non-nocturnal, cost effective are some of the features of zebrafish. Mostly these fishes are found in Eastern India's Ganga River and native to southeastern Himalayan region. They are now available in rivers, ponds and often in aquariums. Its life span is up to 5 years. Zebrafish also has blood, cartilage, bones and teeth. The anatomy of zebrafish resembles the human anatomy about 70% as they are similar to human genes in terms of sequencing.

Sexual maturity of zebrafish will develop around 3 months of age and one pair of adult fish is capable of laying 200 to 300 eggs. The zebra fish embryo develops rapidly within 36 hours of fertilization and within 72 hours they will hatch the eggs. Zebrafish are omnivorous, primarily eats insects. They are considered good for growing in the aquariums. They possess many advantages to the scientists as its genome is fully sequenced and it is easy to understand, observable and testable for developmental behaviors. Zebrafish have ability to generate their heart and lateral line hair cells during their larva stage. Adult zebrafish are able to regenerate different organs like fins, spinal cord, telencephalon and the kidney (Kikuchi K et al., 2011). Ethical Approval was taken by IAEC Committee of SSJ College of Pharmacy and the approval number is 1448/Po/Re/S/11/CPCSEA/SSJCP/2023/05.

Paraquat: Paraquat is a toxic bipiridyl compound. It was discovered in 1950's and used for agricultural purposes (Wesselling C et al., 2005). It is applied by mixing with water and sprayed on weeds to kill them. Paraquat was first described by "Weidel and Russo" in 1882. It is an effective herbicide. The chemical name of Paraquat is N, N-dimethyl-4, 4 bipyridinim dichloride, also known as Methyl viologen. It is an organic compound with chemical formula [C6H7N2] Cl2 (E.T. McKinley et al., 2005). It appears as bright green corrosive liquid with pungent smell. A paraquat salt solution is an adsorbent, surfactant and filler. It is effective when compared with other herbicides like diquat, simazine, linuron, metolachlor and urea herbicides.

Paraquat Dichloride 24% SL herbicide is a unique, fast acting, non-selective for control of most fibrous rooted grass and annual broadleaf weeds. Paraquat is a life threatening poison. The half-life of a paraquat ranges from 16 months to 13 years. Most cases of paraquat poisoning in domestic animals are due to ingesting of paraquat contaminated plants. The toxic effects of paraquat in lungs enhances due to the presence of high concentration of oxygen (Zacharias E et al.,). It can be easily mixed in food, water and other beverages due to its synthetic nature. Multiple methods for detection of paraquat in blood are ultraviolet spectrometry, chromatography and capillary electrophoresis. Fish toxicity studies indicate a threshold toxic concentration for diquat in excess of 10ppm cation and for paraquat in excess of 5ppm cation.

Levodopa: Levodopa is a precursor to dopamine, it is also called as L-DOPA. It is a large neutral amino acid. Levodopa is commonly used for dopamine replacement agent for treatment of Parkinson's disease (Sujith Ovallath et al., 2017. It is mostly used to control Brady kinetic symptoms appeared in Parkinson's disease. Levodopa is prodrug which is converted to dopamine by DOPA decarboxylase and it can cross the blood brain barrier. It is a class of dopamine agonists. Levodopa works similar to the action of dopamine.

Levodopa is only absorbed in the small intestine, which takes the time about 3 hours for absorption. Its chemical name is l-3, 4dihydroxyphenylalanine and was first isolated by Marcus Guggenheim in 1913. It helps to replace the missing dopamine which allows the people for better functioning of brain by increasing the amount of dopamine in brain, and helps to control the

symptoms. Intake of levodopa may cause dizziness, light headache and lose consciousness. Levodopa crosses the blood brain barrier and gets converted into dopamine by decarboxylation. After release it is transported back to the dopaminergic terminals. Its chronic use is associated with dyskinesia, motor fluctuations and hallucinations (Thomas D et al., 2017). The overall bioavailability of L-dopa is only about 10-30% and less than 1% of the oral dose is estimated to reach to the brain unchanged. Dopamine acts on the brain to give the feelings of pleasure, satisfaction and motivation. It is a hydrophilic compound that is rapidly degraded by enzymes within gastrointestinal tract and decarboxylated by DDC during hepatic first pass metabolism.

Preliminary Phytochemical Screening

Preliminary Phytochemical Screening has been conducted on the plant extract to determine the phytochemicals present in the extract (M. Yadav et al., 2014 and Sileshi Dubale et al., 2023).

Experimental design

Fishes are divided into five groups, each group contain 5 fishes (n=5)

Group I (control group) fishes Normal

Group II (Toxic group) Fishes are treated with paraquat (0.04ppm)

Group III (Standard group) - Fishes treated with paraquat (0.04ppm) and levodopa (10 ug /ml)

Group IV (Test group-A) Fishes treated with paraquat (0.04ppm) and *luffa cylindrica* (5 ug/ml)

Group V (Test group-B) Fishes treated with paraquat (0.04ppm) and *luffa cylindrica* (10ug/ml)

Four test groups of fishes were treated individually and subjected to the solution of paraquat, levodopa and luffa cylindrica at a concentration of 0.04ppm, 10ug/ml, 5ug/ml respectively and the remaining control group is treated with vehicle. Once this exposure was given, fishes are kept in a separate beaker accordingly for 30 minutes, after this subjection, the fishes were shifted to another beaker containing fresh water where they were incubated for 15 minutes, then the fish from all treatment groups were shifted individually to examination tank to examine different parameters like locomotors, time spent at the bottom and top of the tank and complete cataleptic time where they were habituated for 5minute. The examination tank contains a fresh aerated water. It consists of a 5-liter tank (30×15×10 m) length x height x weight with number of vertical lines which divided the water tank into 4 compartments. These vertical lines were used to find out the speed of the fish by measuring the time taken by fish to travel from the first vertical lines to last and the horizontal line that indicate the time spent in the upper and lower half of the tank by fish.

All behavioral evaluation done by trial-and-error method measured and observation was done. In addition to this, visual observations were made throughout our experiments and unpredictable like pattern vertical swimming, sideway swimming, and upside- down swimming.

Estimation of Behavioral parameters:

The following behavioral parameters like locomotor activity, complete cataleptic time and time spent near to the bottom of the tank was observed.

Estimation of locomotors of zebra fish: Locomotor is the total distance to travel by the fish from one fixed point to another in examination tank. In this total distance that the fish moved to travel from first vertical line to the last was calculated and the total distance the fish travel under examination was noted down (Bhattacharya KB et al., 2017).

Time spent near the top and bottom of the tank : The time spent by the fish below the horizontal line and above the horizontal line which was drawn on the examination tank was measured at different time intervals. The stressful behavior of the fish under our observation was noted down (McGrath et al., 2008).

Time spent in the light and dark conditions : Light/ dark preference test is a behaviorally validated measure of anxiety in zebrafish. The time spent in the dark and light environment, the latency time to first crossing, and the number of midline crossings were analyzed (Lílian Danielle Paiva Magno et al., 2015).

Statistical Analysis

All values were expressed as mean ± SEM. and data were analyzed by Student's test using the software Graph Pad Prism.

Result & Discussion

Preliminary Phytochemical Investigation: Preliminary phytochemical screening revealed results that methanolic extract of Luffa cylindrica gave positive results for alkaloids, flavonoids, carbohydrates, glycosides, saponins, Phenols, Phytosterols, Diterpenes, and Tannins.

Locomotor activity

dose of 10 ug/mL could increase locomotor activity compared to other doses with a significance value of 0.000 ($p<0.05$).

Tank diving test

Graph 1:

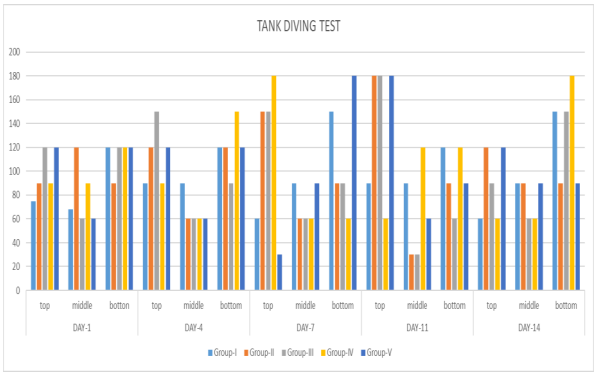
The effect on locomotor activity and behavioral assessment (tank diving test, light and dark test) in adult zebra fish has been extensively studied using paraquat as inducing agent, Luffa cylindrica as test compound and levodopa as a standard.

Table 1: Comparision of various time locomotor activities with various doses of *luffa cylindrica*

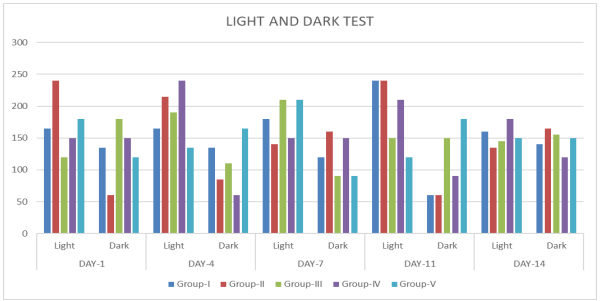
Observation time	Number of Locomotor Activity every 5 minutes (±SD)				
	Group - I	Group - II	Group -III	Group - IV	Group - V
Day - 1	186.2 (±23.60*)	158.02 (±14.03*)	148.08 (±10.23)	170.02 (±13.00*)	153.14 (±03.42**)
Day - 4	184 (±13.02*)	142 (±20.048**)	193 (±18.00)	190 (±18.56***)	210 (±18.26***)
Day - 7	180 (±20.32**)	165 (±18.03***)	200 (±30.23)	223 (±20.35**)	268 (±30.28**)
Day - 11	186 (±23.43**)	153 (±16.32**)	223 (±18.45)	248 (±30.22**)	276 (±31.00**)
Day - 14	190 (±32.36***)	148 (±16.23**)	240 (±15.03)	223 (±18.02***)	286 (±30.28***)

Values expressed Mean± SEM ***p< 0.001, **p< 0.01, *p< 0.05 when compared to Standard group. One-way ANOVA followed by Dunnett's test.

Paraquat appears to mirror the neuropathological, neurochemical, and behavioral aspects of Parkinson's disease (PD) in vertebrae. The rigidity or loss of capacity to move is a key symptom of PD. In this study, giving Paraquat show a decrease in locomotor activity in zebrafish every week, and this condition was possibly due to the influence of temperature and lighting. To determine the effect of Paraquat on locomotor activity, we measured zebrafish motility for five minutes. The decrease in zebrafish motility may be due to a decrease in motor nerve conduction velocity. Evidence of a link between dopaminergic impairment and peripheral motor neuron degeneration in paraquat-induced experimental animals supports this argument. The results are shown standard dose (Levodopa) of 2.5 mg/mL showed significantly different locomotor activity against the toxic group (paraquat) on days 4, 7, 11, and 14. Furthermore, the sample dose (Luffa cylindrica) of 5 ug/mL showed significantly decrease locomotor activity against toxic group on days 1, 4, 7, 11, and 14. Then, the administration of a sample dose (*Luffa cylindrica*) of 10 ug/mL showed significantly different locomotor activity against toxic groups every day. Results shown that the locomotor activity of standard group (10ug/ml levodopa) is similar to the locomotor activity of test



Graph2:



The present study provides the preliminary evidence that the adult zebra fish 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 locomotor activity of Luffa cylindrica on paraquat indused parkinsons adult zebra fish model. As per our knowledge no extensive study has been conducted on zebra fish using these compounds. To validate this model each fish was exposed into different drug concentrations for 15 min.

Degeneration of the substantia nigra occurs in patients with Parkinson disease. This condition results in the disruption of the nigrostriatal pathway and thus decreases the striatal dopamine levels (Nicholas Heng et al., 2023). Unlike dopamine, levodopa can cross the blood-brain barrier (BBB). Levodopa converts to dopamine in both the CNS and periphery.

To increase the bioavailability of levodopa and decrease its side effects, it is often administered in combination with peripheral decarboxylase inhibitors such as carbidopa and benserazide. Dopamine decarboxylase inhibitors prevent the conversion of levodopa to dopamine in the periphery, allowing for more levodopa to cross the BBB (Anouke van Rumund et al., 2021). Once converted to dopamine, it activates postsynaptic dopaminergic receptors and compensates for the decrease in endogenous dopamine.

Our results confirm that when a zebra fish is presented to an unfamiliar environment it shows robust anxiety- like behavioral responses, which were ensured by using the novel tank diving test, and light/ dark transition. Drugs like levodopa reverted anxiety-like behavioral responses, due to high sensitivity, the behavioral and physiological endpoints of the zebra fish can be manipulated. Due to this high sensitivity and manipulation, the novel tank paradigm possesses a great potential for use in the screening of novel compounds of possible therapeutic value. behavioral endpoints such as thigmotaxis (staying closed to the walls) decreased exploration and freezing found in rats indicate anxiogenic behavior is now applied to zebra fish model of anxiety. Centre and peripheral ratio of rodents in open field test and top: bottom ratio in novel tank test is similar kind of behavioral endpoints. Studies have indicated that similar environmental conditions cause anxiety like behavior both in rodents as well as in zebra fish.

Fish can also form special memories just like the rodents and use them to guide themselves and establish safe zones in novel environment.

The results showed that the behavioral responses at the top middle and bottom of the tank by the zebra fishes of the test group (*Luffa cylindrica*) is significant to that of similar standard group (Levodopa). A similarity has also been found between the hyper arousal behavior found in rodents in dangerous situations and erratic movements shown by Zebra fish insignificant decrease in locomotor activity was observed at higher dose. Studies have indicated that similar environmental conditions cause anxiety like behavior both in rodents as well as in zebra fish (Adam Stewart et al., 2011). Fish can also form special memories just like the rodents and use them to guide themselves and establish safe zones in novel environment. A similarity has also been found between the hyper arousal behavior found in rodents in dangerous situations and erratic movements shown by Zebra fish in novel tank. Thus, zebra fish could be considered as useful animal model for the study of anxiety and screening of new drugs on the basis of locomotor activity and behavioral endpoints of zebra fish.

The light dark preference test used for rodents is also a useful paradigm for investigating anxiety like behavior in Zebrafish. Studies showed that toxic drug paraquat increased the exploratory behavior and time spent in white compartment while standard drug levodopa caused the opposite effect. The results show that the exploratory behavior and the time spent in the light and dark compartments of the standard drug (levodopa) is similar to the test group b (Luffa cylindrica10ug/ml). There are several characteristics which make Zebra fish an important test subject which could prove useful in gaining a greater understanding of neuro pharmacological mechanisms in mammals and facilitate behavior-based drug discovery. Since zebra fish have robust physiological responses and quantifiable behavioral and neuropathological phenotypes analogous to humans, several beneficial properties make zebra fish a promising alternative to mammalian model. Low maintenance cost and rapid life cycle of zebra fish makes easy to maintain in large number of fish in small area which is important for large scale behavioral studies. Zebra fish readily acclimations to new environments, is constantly active and very little disturbed by the presence of Observers (Jason A et al., 2007).

These qualities make zebra fish an excellent species choice for behavioral study. Zebra fish has similarity in basic organization of brain components to that of humans which make it useful in the study of brain disorders. The zebra fish has been used in the study of neurodegenerative diseases such as Parkinson's disease, Huntington's and Alzheimer's diseases. Reported the use of zebra fish model for evaluating the Anti Parkinson's effect of methanol extract of the leaves of Luffa cylindrica. The basic and complex brain phenomena as well as endocrine mechanisms of zebra fish and mammals are substantially homologous. Zebra fish model enables greater insight into neural mechanism associated with anxiety related disorders since it possesses all the classical vertebrates' neurotransmitters (Allan V Kalueff et al., 2015). Much like rodents, zebra fish has the ability to learn through classical conditioning. It also offers an alternative and efficient mode of drug delivery via the gills. Zebra fish can serve, therefore, as an inexpensive and potentially high throughput model for Anti Parkinson's activity and behavioral assessment of medicinal plants for drug development.

Conclusion

In view of the above findings of this study we conclude that the results suggest that exposure of zebrafish with methanolic extract of Luffa cylindrica leaves produces the expected changes in the behavior of the zebrafish. The locomotor activity, novel tank driving test and Light and dark transition test are successfully used to measure behavioral responses. Hence, the zebrafish is an excellent laboratory model organism for high throughput screening. The novel tank test demonstrated the behavioral and pharmacological tool for attainable goal of the zebrafish. Therefore, zebrafish serves as model for behavioral and psychopharmacological screening of medicinal plant for development of the drug. Zebrafish can be used as alternative model for the assessment behavioral activity in biomedical research by using novel chemical compounds.

Disclosure

Ethics approval and consent to participate

Ethical Approval was taken by IAEC Committee of SSJ College of Pharmacy and the approval number is 1448/Po/Re/S/11/CPCSEA/ SSJCP/2023/05.

Availability of data and materials

Data are available upon reasonable request

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Competing interests

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Authors' contributions

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