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Neuroprotective effect of Kojic acid in Rotenone - induced Parkinsonism in a Zebrafish Model

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Abstract

Parkinson's disease is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra, resulting in motor impairments such as bradykinesia, tremors, and postural instability. The zebrafish (*Danio rerio*), with 87% genetic closeness to humans, serves as an exemplary model for investigating neurodegeneration. Kojic acid is a natural metabolite produced by fungi *Aspergillus oryzae* and has been reported for antimicrobial, antioxidant, anti-inflammatory and anticonvulsant properties. The present study investigates the neuroprotective effect of Kojic acid in a zebrafish model of Parkinson's disease. Zebrafishes were divided into six experimental groups namely Normal, Positive control, Standard, and Kojic acid treated group. Parkinsonian symptoms were induced in the zebrafish using the neurotoxin rotenone (5 µg/L). Following induction, the fishes were treated with standard (Kapikacchu, *Mucuna prunens* extract, 20 mg/L) and kojic acid (5, 10, and 20 mg/L) for 28 days, with the medium being changed every 48 hours to maintain the concentrations. Behavioural analysis was conducted using ANY-MAZE software. On the 29th day, the zebrafishes were euthanized and their brains were extracted for the assessment of biochemical parameters and histopathology. The study revealed that zebrafish exposed to 10 mg/L of Kojic acid exhibited a notable enhancement in locomotor activity relative to the positive control group, indicating Kojic acid as a potential option in the treatment of Parkinson's disease.

Keywords: Parkinson's disease, Kojic acid, Rotenone, Zebrafish, locomotory impairment, neurodegeneration

Introduction

Parkinson's disease is a progressive neurodegenerative disorder marked by the loss of dopaminergic neurons in the substantia nigra, leading to movement issues such as pill-rolling tremors (tremors on the hand), akathisia (an inability to remain seated), rigidity, akinesia, bradykinesia, unstable posture (stooped), absence of rhythmic arm swinging with leg movement, sialorrhea, oculogyric crisis (fixed gaze for a variable duration), mental depression, restlessness, seborrhoeic dermatitis, and paralysis facial muscles. While a cure for Parkinson Disease (PD) remains elusive, various treatment strategies are designed to manage symptoms and slow down the progression of the disease (1). One approach that has gained attention in

recent years is using phytochemicals, naturally occurring compounds found in plants, to treat Parkinson's disease. Phytoconstituents from natural source have shown promising potential as therapeutic agents for Parkinson's disease. These phytochemicals possess various pharmacological properties, including antioxidant, anti-inflammatory, neuroprotective, and anti-aggregation effects. Moreover, they have been discovered to regulate PD-related pathways and provide neurotrophic support. These phytochemicals are naturally sourced and may be derived from medicinal plants, plant products, and secondary products (2). Kojic acid (5-hydroxy-2-hydroxymethyl-1–4-pyrone) is a naturally occurring substance that belongs to the group of fungal metabolites, which are produced by specific strains of Aspergillus fungi, such as *Aspergillus oryzae*. They use it in beauty products to whiten the skin, fade age spots and other skin discolorations, including melasma, and for anti-aging properties. Notably, apart from the cosmetic uses, kojic acid has antibacterial, antifungal, and anti-inflammatory properties. In addition, this compound serves the purpose of an antioxidant, and it helps in the prevention of diseases caused by oxidative stress (3). Kojic acid and its derivative revealed the neuroprotection activity against Alzheimer's disease, and has radio protective, anti-proliferative effects (4).

Materials and Methods

Chemicals and reagents

Rotenone (R8875-1G with CAS number 83-79-4) was obtained from Sigma-Aldrich, Kojic acid (CAS number 501-30-4) was obtained from Yucca enterprises, DMSO (Dimethyl sulfoxide), 0.1M,1.15 % KCl, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), Acetyl Thio Choline (ATC) chloride, hydrogen peroxide, Nitro Blue Tetrazolium (NBT), Tris (hydroxymethyl) methylamine, Sodium chloride, NBT, 2 µM riboflavin, and Ethylene di amine tetra acetic acid (EDTA), Dipotassium phosphate, potassium dihydrogen phosphate, Disodium phosphate, sodium dihydrogen phosphate

Experimental animals

Adult Zebrafish (short-fin, 6-8 months old the ratio of male and female was nearly 1:1) were purchased from 777 Aquatics, Madhya Pradesh, India. The adult zebrafish were housed in rectangular tanks of height 15 cm, 30 cm length, and 16 cm width with a temperature maintenance of 25 ± 2°C. These tanks were then filled with 3L of tap water with a pH range of 7. 0 - 8. 0. For artificial lighting, overhead fluorescent light tubes were attached; the fishes were exposed to a 14/10 light and dark cycle and fed with commercial pelleted fish feed twice daily. Zebrafishes were assimilated to laboratory environment at least for 10 days. The experiment protocol was approved by the Institutional Animal Ethical Committee of M. S. Ramaiah University of Applied Sciences, Faculty of Pharmacy (Certificate No: XXVII/ MSRFPH/COG/UG-GP-26/13.02.2023).

Acute toxicity studies

The acute toxicity study followed OECD 203 guidelines (5). Seven healthy fishes were placed in varying concentrations of kojic acid (12.5, 25, 50, and 100 mg/L) to assess the acute toxicity levels for 96 hours. Throughout this period, observations on mortality were recorded at 24, 48 and 96 hour intervals, focusing on determining the concentrations at which 50 percent of the fish showed signs of toxicity (Fig. 1).

Figure 1. Glass Tank containing 7 zebrafishes for acute toxicity studies

Experimental protocol

Zebrafish (n=7) were divided into six experimental groups: Normal, Positive control, Standard (Kapikacchu, standardized extract of Mucuna pruriens)), Kojic acid 5 mg/L, Kojic acid 10 mg/L and Kojic acid 20 mg/L (Table 1). To induce Parkinson's disease in zebrafish, 5 µg/L rotenone (Sigma R8875) was dissolved in a volume of water equal to 3 Liters. The rotenone concentration was maintained by refreshing the water every 2 days. Tap water temperature was maintained at 25–27 °C under a 14 hour light and 10 hour dark cycle. Kojic acid at 5mg/L, 10mg/L, and 20mg/L were administered with rotenone consecutively for twenty-eight days (6). During the 28 days study recordings were made on the 7th, 14th, 21st, and 28th day to observe the locomotory activity of the fish. On the 28th day, a series of tests including Novel Tank Diving test, Light/Dark Preference Test, and Open Field Arena Test were conducted using ANY-Maze software.

Table 1. Animal grouping and dosing regimen

Sl. No	Groups	Treatment	Dose
1	Normal	Water	-
2	Positive control	Rotenone + DMSO	5 µg/L
3	Standard	Kapikacchu	20mg/L
4	Test 1	Kojic acid	5mg/L
5	Test 2	Kojic acid	10mg/L
6	Test 3	Kojic acid	20mg/L

Novel tank diving test (NTD)

NTDT was performed to evaluate anxiety-like behavior in zebrafish. One prevalent non-motor symptom of Parkinson's disease (PD) that can have a detrimental effect on both motor disability and quality of life is anxiety. The test consists of acclimating zebrafish to the experimental room and then carefully transferring them to a new type of tank of height 15 cm, 30 cm length, and 16 cm width with a white background to enhance the contrast of the video recording. The NTDT was carried out, which assesses locomotor activities like total distance travelled (7). The fishes initially stay at the bottom of the tank and then gradually increase their vertical swimming as they explore the area. The time spent in each area of the pool, the total distance traveled and number of transitions to the top zone were determined using the ANY-Maze video tracking software. A gradual increase in exploration indicates a reduction in anxiety.

Light/dark preference test (LDPT)

The light/dark preference test is commonly used to assess scototaxis behavior. Zebrafish are known to show 'scototaxis' behavior and hence tend to avoid areas that have light. The tank of height 15 cm, 30 cm length, and 16 cm in width was placed on a flat surface, with equal division along the vertical plane, which was divided at the halfway mark with a black and white color. Subsequently the fish was put in light for 1-2 min with the sliding door closed. The sliding door was then opened, and the video recording was continued further for 5 minutes with the help of ANY-Maze video tracking system and data acquired was analyzed (8).

Open field arena test (OFAT)

The motor dysfunction of adult zebrafish was accessed using the open field test. The apparatus consisted of a 5 liter square tank with dimensions of 15 cm height, length, and width filled with 3 Liters of tap water. Zebrafish typically swim back and forth along the length of the tank, and this behavior was used to evaluate the locomotor movement. In Open field arena, the entire tank was virtually divided into 9 equal parts (every 7 centimeters) in length by 3 vertical and 3 horizontal lines. The locomotor activity was recorded for 5 minutes using the ANY-MAZE system that recorded the time taken by zebrafishes to cross all zones, the total distance traveled and the mean velocity (7).

Biochemical parameters

After assessing the behavioral patterns, zebrafishes were euthanized on the 29th day by immersing them in ice-cold water for 10 minutes until opercular movements ceased (Fig.2, 3). After that, the whole brain was removed using spring scissors (Fig. 4) and ophthalmic forceps to assess various biochemical parameters. Whole brains were homogenized in 5 ml ice-cold 0.1M Potassium Phosphate buffer (pH 7.4) with 1.15% KCl using tissue homogenizer and then centrifuged at 1000 rpm to separate the supernatant. The supernatant was used to determine acetylcholinesterase (AChE), Catalase, Superoxide Dismutase (SOD), and reduced glutathione (GSH) (9) (10).

Figure 2. Euthanized fish

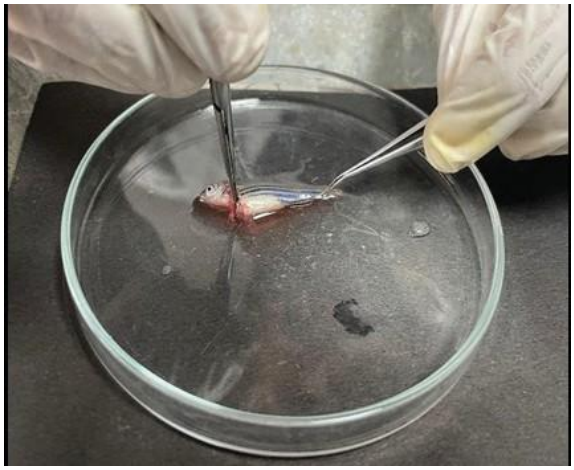


Figure 3. Dissection of brain

Figure 4. Isolated brain

Histopathological study

Tissue samples were collected and stored in 10% formalin. The tissue sections were stained with hematoxylin and eosin, with minor modifications. Coat the slices with DPX medium, then put a cover glass over them, and observe under a light microscope for any histopathological change (11).

Statistical analysis

The statistical results for all experimental groups or parameters are presented as mean ± S.E.M. (Standard Error of Mean). Using Analysis of Variance (ANOVA) to compare the statistical **results.**

Results and Discussion

Acute toxicity of Kojic acid in zebrafish

Acute toxicity testing was conducted following OECD guidelines 203. Fish exposed to various concentrations (12.5, 25, 50, and 100 mg/L) of Kojic acid for 96 hours displayed no behavioural changes or mortality. The result showed that Kojic acid was safe at 100 mg/L concentration.

Anti-Parkinson activity

The zebrafishes were divided into six groups of seven each. The locomotor activity of the zebrafishes in all groups were recorded for 5 mins as track plots, heat map and the speed chart by ANY MAZE software and shown in figure 5.

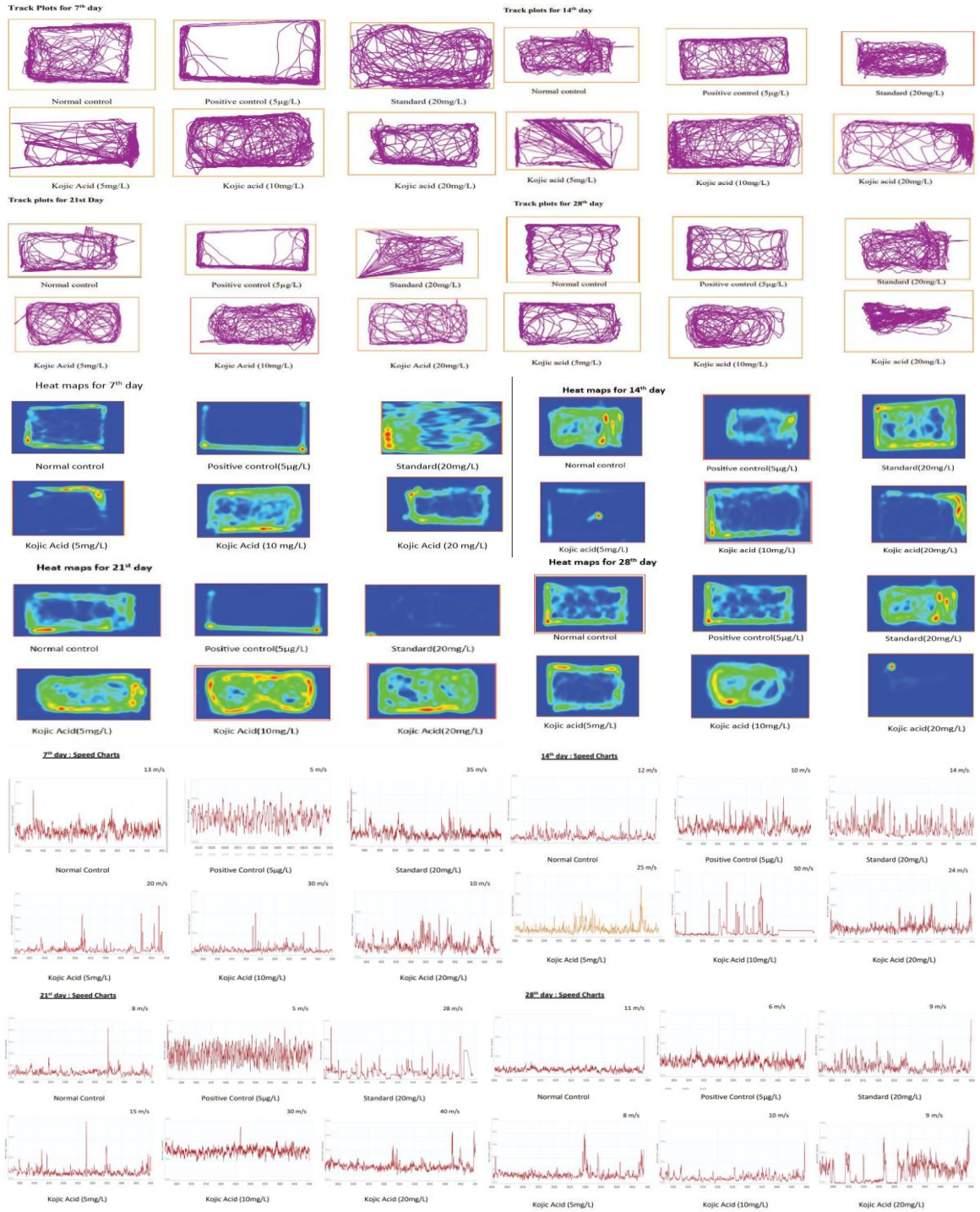


Figure 5 Track plot, heat maps and speed charts of zebra fishes during thier assesment of locomotor activity

Novel tank diving test

The track plots, heat maps and speed chart of locomotor activity of zebrafishes were shown in figure 6-8. The total distance travelled and the number of transitions to the top zone was shown in Table 2 &3.

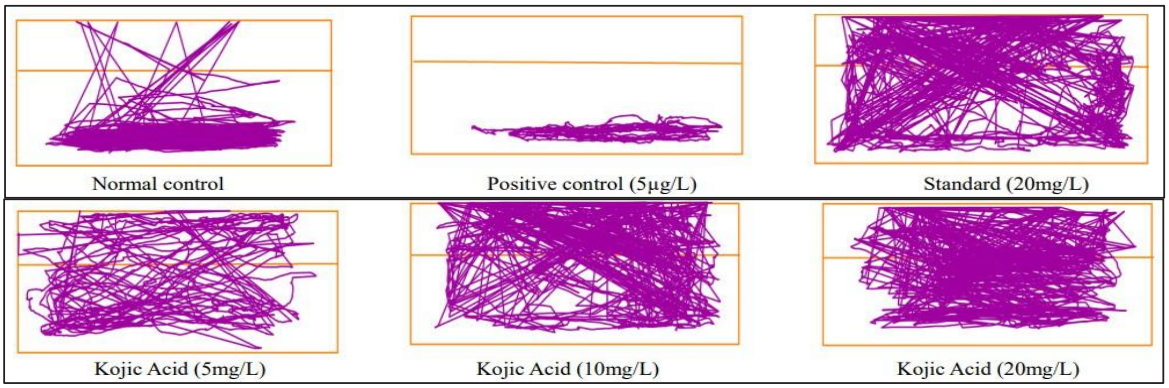


Figure.6: Track plot representing total distance travelled and number of transitions to the top zone in Novel Tank Diving test

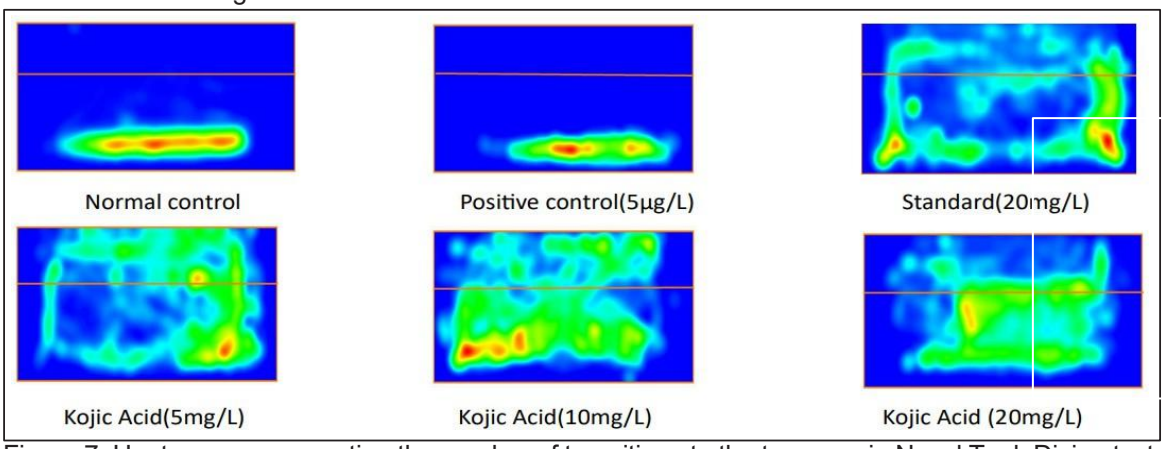


Figure.7: Heat maps representing the number of transitions to the top zone in Novel Tank Diving test

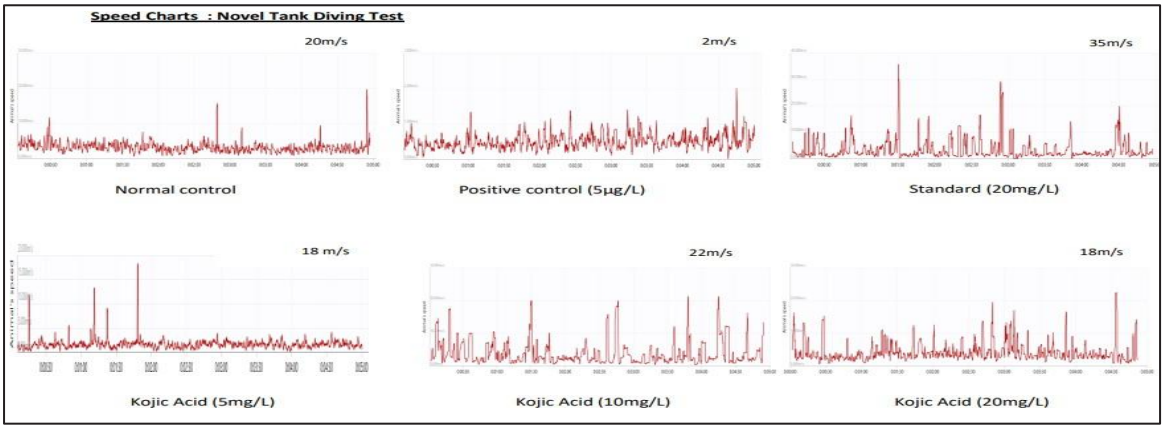


Figure.8: Speed chart of zebrafishes in Novel Tank Diving Test

Table 2. Total distance travelled in NTDT

Sl no	Group	Total distance travelled (meters)
1	Normal	11±0.44****
2	Positive Control	6.8±0.37
3	Standard	11.4±0.24****
4	Kojic Acid 5 mg/L	8.4±0.24**
5	Kojic Acid 10 mg/L	10.8±0.24****
6	Kojic Acid 20 mg/L	9.8±0.24****

Data represents mean ± S.E.M. n=6. ANOVA. **p<0.01 and ****p < 0.0001 compared with positive group

Table 3. Number of transitions to the top zone in NTDT

Sl no	Group	Number of transitions to the top zone
1	Normal	9.4±0.6*
2	Positive Control	7.8±0.37
3	Standard	15.4±0.24****
4	Kojic Acid 5 mg/L	10.6±0.4***
5	Kojic Acid 10 mg/L	13.4±0.37****
6	Kojic Acid 20 mg/L	12.6±0.37****

*Data represents mean ± S.E.M. n=6. ANOVA. *p<0.05, ***p < 0.001, ****p < 0.0001, compared with positive group*

Light/dark preference test

The track plots, heat maps and speed chart of locomotor activity of zebrafishes were shown in figure 9-11. The number of transitions to light zone, the time spent both in light and dark zones were represented in Table 4, 5 & 6.

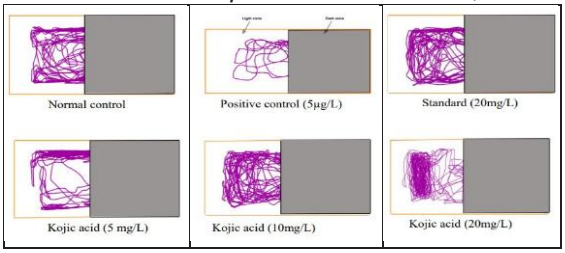


Figure.9: Track plot representing number of transitions and time spent in the light zone in Light/Dark Preference Test

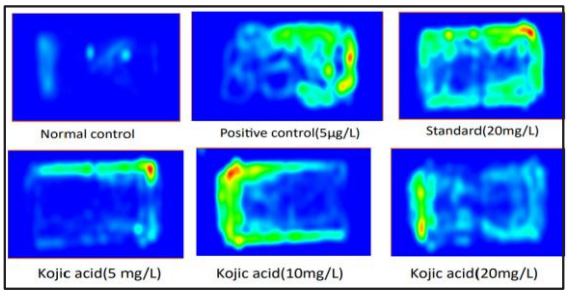


Figure.10: Heat Maps representing number of transitions and time spent in the light zone in Light/Dark Preference Test

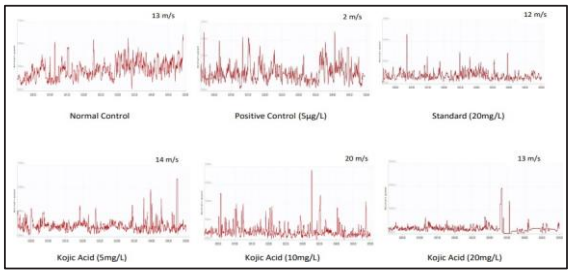


Figure.11: Speed Charts in the light zone in Light/Dark Preference Test

Table 4. Number of transitions to the light zone

Sl no	Group	Number of transitions to the light zone
1	Normal	18.8±0.48****
2	Positive Control	6.6±0.24
3	Standard	20.8±0.48****
4	Kojic Acid 5 mg/L	10.6±0.24****
5	Kojic Acid 10 mg/L	14.4±0.24****
6	Kojic Acid 20 mg/L	15.8±0.48****

Data represents mean ± S.E.M. n=6. ANOVA. ****p < 0.0001 compared with positive group.

Table 5. Time spent in light zone

Sl no	Group	Time spent in light zone (seconds)
1	Normal	188±4.89****
2	Positive Control	56±2.44
3	Standard	238±4.89****
4	Kojic Acid 5 mg/L	88±4.89****
5	Kojic Acid 10 mg/L	164±2.244****
6	Kojic Acid 20 mg/L	144±2.44****

Data represents mean ± S.E.M. n=6. ANOVA. ****p < 0.0001 compared with positive group.

Table 6. Time spent in dark zone

Sl no	Group	Time spent in dark zone (Seconds)
1	Normal	94±2.44****
2	Positive Control	235.2±2.4
3	Standard	145±2.23****
4	Kojic Acid 5 mg/L	173±2****
5	Kojic Acid 10 mg/L	117.4±3.12****
6	Kojic Acid 20 mg/L	151±2.44****

Data represents mean ± S.E.M. n=6. ANOVA. ****p < 0.0001, compared with positive group.

Open field arena test

The track plots, heat maps and speed chart of locomotor activity of zebrafishes were shown in figure 12-14. The total distance travelled and the time taken to cover all zones were represented in Table 7 & 8.

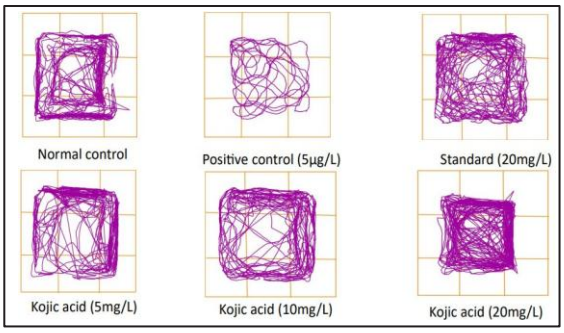


Figure 12. Track plot representing total distance traveled and number of transitions to all zones in Open Field Arena Test

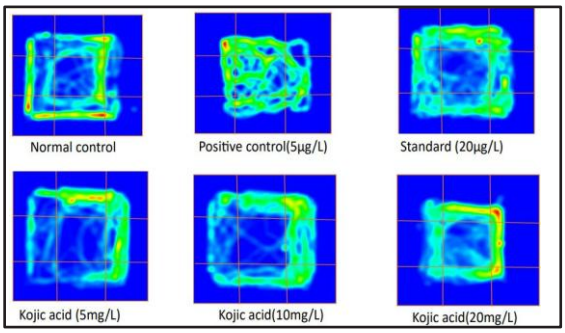


Figure 13. Heat Maps representing total distance traveled and number of transitions to all zones in Open Field Arena Test

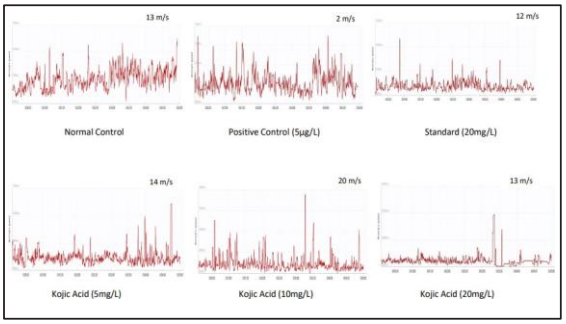


Figure 14. Speed charts of Zebrafishes in Open Field Arena Test

Table 7. Total distance travelled in the open field arena test

Sl no	Group	Total distance travelled (meters)
1	Normal	10.2±0.37****
2	Positive Control	6±0.44
3	Standard	17.4±0.24****
4	Kojic Acid 5 mg/L	9.4±0.24****
5	Kojic Acid 10 mg/L	14.8±0.37****
6	Kojic Acid 20 mg/L	11.2±0.739****

Data represents mean ± S.E.M. n=6. ANOVA. ****p < 0.0001, compared with positive group.

Table 8. Time taken to cover all the zones in the open field arena test

Sl no	Group	Time taken to cover all the zones in the open field (seconds)
1.	Normal	10.2±0.37****
2	Positive Control	6±0.44
3	Standard	17.4±0.24****
4	Kojic Acid 5 mg/L	9.4±0.24****
5	Kojic Acid 10 mg/L	14.8±0.37****
6	Kojic Acid 20 mg/L	11.2±0.739****

Data represents mean ± S.E.M. n=6. ANOVA. ***p<0.001, ****p < 0.0001, compared with positive group

Biochemical parameters

Various antioxidants levels were measured in the brains of zebrafishes of all groups and were shown in table 9.

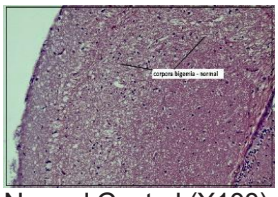
Table 9. Effect of Kojic acid on AChE, SOD, Catalase, and reduced Glutathione levels in rotenone-induced PD in zebrafish

Sl No.	Treatment Groups	AChE levels (Umol/sch.h/mg of protein)	SOD levels (Umin/mg of protein)	CAT Activity (Umol/min/mg of protein)	GSH levels (Umol/mg of protein)
1	Normal Control	14.61±1.35**	2.45±0.21***	2.68±0.24**	12.13±1.10**
2	Positive control (5µg/L)	8.26±0.82	1.29±0.12	1.54±0.14	6.28±0.62
3	Standard (20mg/L)	14.54±1.35**	2.08±0.19*	2.46±0.21*	11.60±1.08**
4	Kojic Acid (5mg/l)	13.98±1.32*	2.16±0.19*	2.51±0.23*	11.23±1.08**
5	Kojic Acid (10mg/L)	14.48±1.39**	2.24±0.23**	2.56±0.24**	11.89±1.11**
6	Kojic acid (20mg/L)	13.65±1.32*	2.06±0.18*	2.39±0.22*	11.13±1.17*

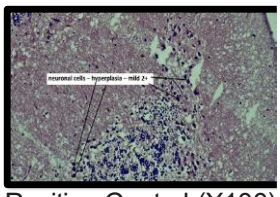
Data represents mean ± S.E.M. n=6. ANOVA. ***p<0.001, ****p < 0.0001, compared with positive group

Histopathological studies

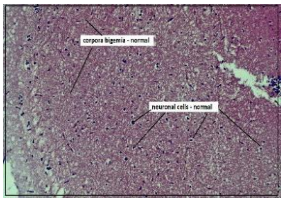
The brain histopathology of zebrafishes from different groups were shown in figure 15.



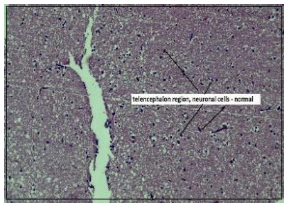
Normal Control (X100)



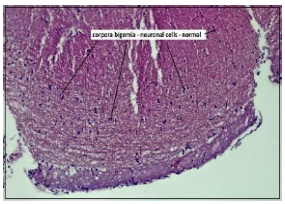
Positive Control (X100)



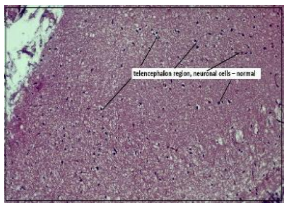
Standard (X100)



Kojic acid Treated (5mg/L) (X100)



Kojic acid Treated (10 mg/L) (X100)



Kojic acid Treated (20 mg/L) (X100)

Figure 15. Histopathology of Zebrafishes brain to positive control (rotenone-induced,

Discussion

Parkinson's disease is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, a brain region pivotal for motor control. Rotenone, an intricate lipophilic neurotoxin, breaches through both the blood-brain barrier and cell membrane without requiring energy. In zebrafish, inhibition of mitochondrial complex I by rotenone leads to ROS increase that causes damage of mitochondria and results in Parkinson's-like symptoms (12).

Numerous research concerning drug screening, neurotransmission, and neurodevelopment employ zebrafish as a model organism due to their 87% genetic, physiological, and anatomical similarities with humans. Additionally, its unique neurological system contains dopaminergic neurons that are comparable to those found in the adult human brain's substantia nigra (12). The zebrafish's locomotor activity was tracked on 7th, 14th, 21st and 28th day. On 7th day, rotenone-induced group zebrafishes showed reduced locomotor activity and among the treatment groups, Kojic acid (10 mg/L) showed an increased locomotor activity. On the 14th day, fishes treated with Kojic acid (10 mg/L) exhibited an increase in locomotion with a maximum speed of 50m/s when compared 10m/s). On the 21st day, Kojic acid (10 mg/L) treated zebrafishes displayed an increased loc-

omotor activity and travelled at a speed of 30m/s higher than the positive group. On 28th day, the Kojic acid (10 mg/L) treated group showed increased locomotor activity and travelled at a speed of 10 m/s in contrast to positive control (6m/s) The in- creased speed and locomotor activity of 10mg/L of Kojic acid was also well supported by the re- corded heat map chart.

The behavioural analysis was carried out by Novel tank diving test, Light/dark preference test and Open Field Arena test. Anxiety is a common non-motor symptom of PD (13). NTD is performed to assess anxiety-like behaviour, total distance travelled and the number of transitions to the top zone. The rotenone treated group exhibited reduced movement, extended bottom-dwelling period and showed a delay in exploring the top zone. When compared to other treatment groups Zebra fish treated with Kojic acid (10 mg/L) showed an increased movement towards the top zone. Similarly, the heat map of Kojic acid (10 mg/L) indicated that Zebra fishes preferred to stay at the top zone and the speed chart showed a peak at 22m/s. More top entries indicate lower anxiety level demonstrating the anti-Parkinson effect of Kojic acid.

In the Light/Dark Preference Test, the time spent in the light/dark zone and the number of transitions to the light zone were measured. Zebrafishes exposed to rotenone showed scototaxis behaviour and spent more time (235 seconds) in the dark zone. Zebrafish treated with kojic acid at doses of 5 mg/L and 20 mg/L spent more time in the dark than in the light zone (173 and 151 seconds, respectively), indicating a higher level of anxiety than those given with a dose of 10 mg/L (117 seconds). In contrast to the positive group (56 seconds), zebrafishes spent 164 seconds in the bright zone, demonstrating the anti-anxiety properties of kojic acid. The speed chart of 10 mg/L dose showed a peak at 15 m/s and increased transitions to the light zone.

Open field test is an experimental procedure used to measure the general locomotor activity levels, anxiety, and exploratory willingness of animals. In this test, locomotory functions such as total distance travelled, and time taken to cover all zones in the open field arena were measured. The normal group exhibited uniform movement both in the central region and at the periphery, while the positive control zebrafish remained in the center of the tank and took a longer period to cross each zone (115 seconds). Among the treatment groups, the time taken by Kojic acid (10 mg/L) to cross all the nine zones was found to be 55 seconds which was a short duration when compared to the other two treatment groups (kojic acid 5 mg/L- 75 seconds, Kojic acid 20mg/L -76 seconds). The speed chart of Kojic acid (10 mg/L) showed increased motor function with a speed of 21 m/s when compared to positive control (1.5 m/s). The total distance travelled by zebrafishes was tracked for normal, positive control, standard drug-treated, kojic acid treated (5 mg/L, 10 mg/L, and 20 mg/L) groups and was found to be 10.2, 6, 17.4, 9.4, 14.8 and 11.2 meter respectively. The above result indicates that kojic acid 10 mg/L showed an increase in total distance traveled, speed, and time taken to cover all zones in the open field arena.

Various biochemical parameters like acetyl cholinesterase (AChE), Superoxide Dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH) were determined in zebrafishes brain homogenate. These are the biomarkers of antioxidant property and their levels determine the oxidative damage to the brain (8). In rotenone induced group, the level of AChE, SOD, CAT and GSH were significantly reduced. Acetylcholinesterase is an essential cholinergic enzyme found at postsynaptic neuromuscular junctions in muscles and nerves. The main role of the enzyme is the termination of neuronal transmission and signaling between synapses through the breaking down of acetylcholine into acetic acid and choline, by preventing nearby receptors from getting activated (13). Kojic acid (10 mg/L) significantly (**$p < 0.01$) increased AChE level in comparison to positive control.

Superoxide dismutase is an antioxidant enzyme that catalyzes conversion of the superoxide radicals to oxygen and hydrogen peroxide. Thus, the levels of SOD would be representative speak for the status of oxidative stress and detoxifying ability of the brain toward ROS, which gives insight into neuroprotective capacity (14). Compared to all concentrations, kojic acid at concentrations of 10 mg/L significantly (**p < 0.01) elevated SOD activity compared to the positive control. Catalase is another crucial antioxidant enzyme, which breaks down hydrogen peroxide the harmful byproduct of cellular metabolism in the body, into water and oxygen. It participates in decreasing oxidative stress and reflects the general oxidative damage to the brain and its whole antioxidant capacity. This means that high concentrations of an antioxidant enzyme hydrogen peroxide can react rapidly with highly toxic hydroxyl radical (-OH) (15). When compared to the positive control, Kojic acid at 10 mg/L dose significantly (**p < 0.01) increased CAT activity.

Glutathione is an antioxidant tripeptide formed through dehydration condensation resulting from cysteine, glycine's, and glutamic acid. Hence, it takes part in redox reactions that secure nerve cells against oxidative damage resulting from oxygen free radicals. A low GSH/GSSG ratio caused by oxidative stress is related to mitochondrial disorder and plays a core role in neuroinflammation and neurodegeneration during Parkinson's disease (16). Kojic acid (10mg/L) was able to reverse inhibition of GSH levels and increased the activity by 11.89 µmol/mg of protein respectively.

The histopathology of normal Control Zebra fish brain showed a normal morphology of corpora bigemia and cerebellum neuronal cells and no abnormalities was observed whereas zebrafish brain treated with positive control (rotenone) showed inflammations and apoptosis. The standard and kojic acid treated zebrafishes brain showed normal morphological structure of Corpora bigemia, cerebellum and telencephalon neuronal cells with reduced inflammation supporting the neuroprotective effect of kojic acid.

When compared to lower and higher doses, mid dose (10mg/L) showed better anti-Parkinson effect. The increased potency of the 10 mg/L dose may be likely due to combination of factors. Perhaps moderate doses maximize the hormesis effect, mediating the best therapeutic response, without the suboptimal or toxic effects present at the extremes. Higher doses might result in toxicity, receptor saturation or desensitization that diminishes effectiveness, while lower doses could not attain adequate biological activity. Besides, the mid dose could possibly have best pharmacokinetics, which is essential for an effective absorption, distribution and metabolism.

Conclusions

The findings of this study demonstrate that Kojic acid mitigates rotenone-induced neurobehavioral alterations in zebrafish. The study provided strong scientific evidence that Kojic acid reduces anxiety-like behaviour, locomotor impairment, and the onset of Parkinsonism and improves motor function. These results support the potential of Kojic acid as a neuroprotective agent in PD models and further investigation into its therapeutic applications for Parkinson's disease.

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